# *Microfluidic Chips for Integrated DNA Assays**

C. H. Mastrangelo*

Biochemical Technologies
Sullivan Park Research Center
Corning, NY

*Center for Wireless Integrated Microsystems
An NSF Engineering Research Center
University of Michigan
Ann Arbor, MI

# *Outline*

- Motivation
- DNA Assay Toolkit
- Integrated Microfluidic Systems
- Microfluidic Component Implementation
- Summary

# *Why DNA Assay Chips ?*

- Genomic DNA is a **long linear polymer** containing the basic blueprint for a given organism (about 3.5 billion units (ACGT) for humans ~ 7 Gb)
- Information organized as **Genes** identifying specific organism feature
- **Genes** provide vital information for :
  - **Probability of hereditable diseases (~400 known)**
  - **Early cancer detection (BRC1,2 p53 apoptosis regulatory gene)**
  - **Pharmacogenomics (genetically targeted drug treatments)**
  - **Organism identification (food science, agriculture, livestock mgmt)**
- **HGenome** has about **~30,000 genes:** ***THOUSANDS OF TESTS PER INDIVIDUAL***
- DNA assays are universal (animal, plant): ***VIRTUALLY UNLIMITED UTILITY***

***CAN WE USE MINIATURIZATION TECHNOLOGIES TO REDUCE COST AND INCREASE ACCESSABILITY OF DNA ASSAYS ?***

# DNA Structure

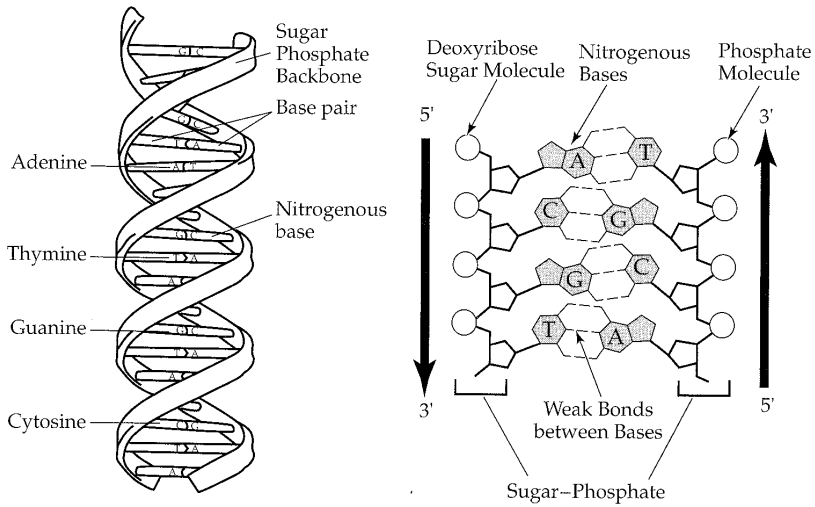
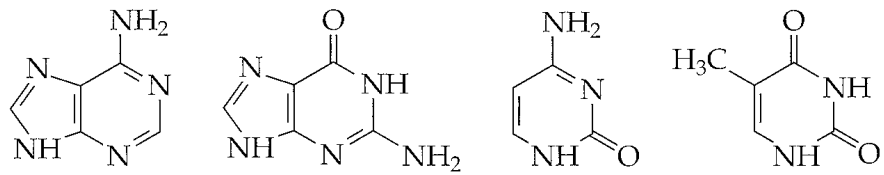


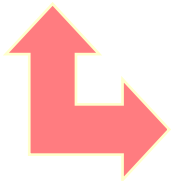

**FIGURE 2.13** DNA forms a double-stranded helix with a uniform radius and angle of twist. The sugar–phosphate backbone forms the outer shell of the helix. The two strands of DNA run in opposite directions. Bases face toward each other and form hydrogen bonds. The types of base pairs found in DNA are restricted to those shown in the figure. (Modified from http://www.nhgri.nih.gov/DIR/VIP/Glossary/Illustration/basepair.html.)

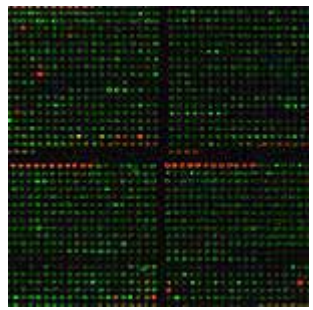



The nitrogenous bases adenine and guanine are composed of two rings. The corresponding nucleotides are called *purines*. The bases of the other two nucleotides, dCMP and dTMP, have single rings and are called *pyrimidines*. The name for the nitrogenous base of each DNA monomer identifies the monomer itself. Thus, a DNA strand is composed of monomers identified as adenine (A), guanine (G), thymine (T), and cytosine (C).

# *Two Major DNA Assay Methodologies*

- **Gene Fingerprinting Method**:
  - Look for match of DNA sample to known target gene using “probes”
  - Positive/negative answer
  - Good for detecting identifiable genes and genetic differences or single nucleotide polymorphisms (SNPs) in well known organisms
  - Can make arrays of matching test “pixels”
  - Most common embodiments are parallel genotyping microarrays (commercially available)
    - **Arrayed hybridization** “selective binding” arrays with large number of short (10-50mer oligo) probes
    - **Parallel multicolor or arrayed amplification** (selective flanking gene markers)

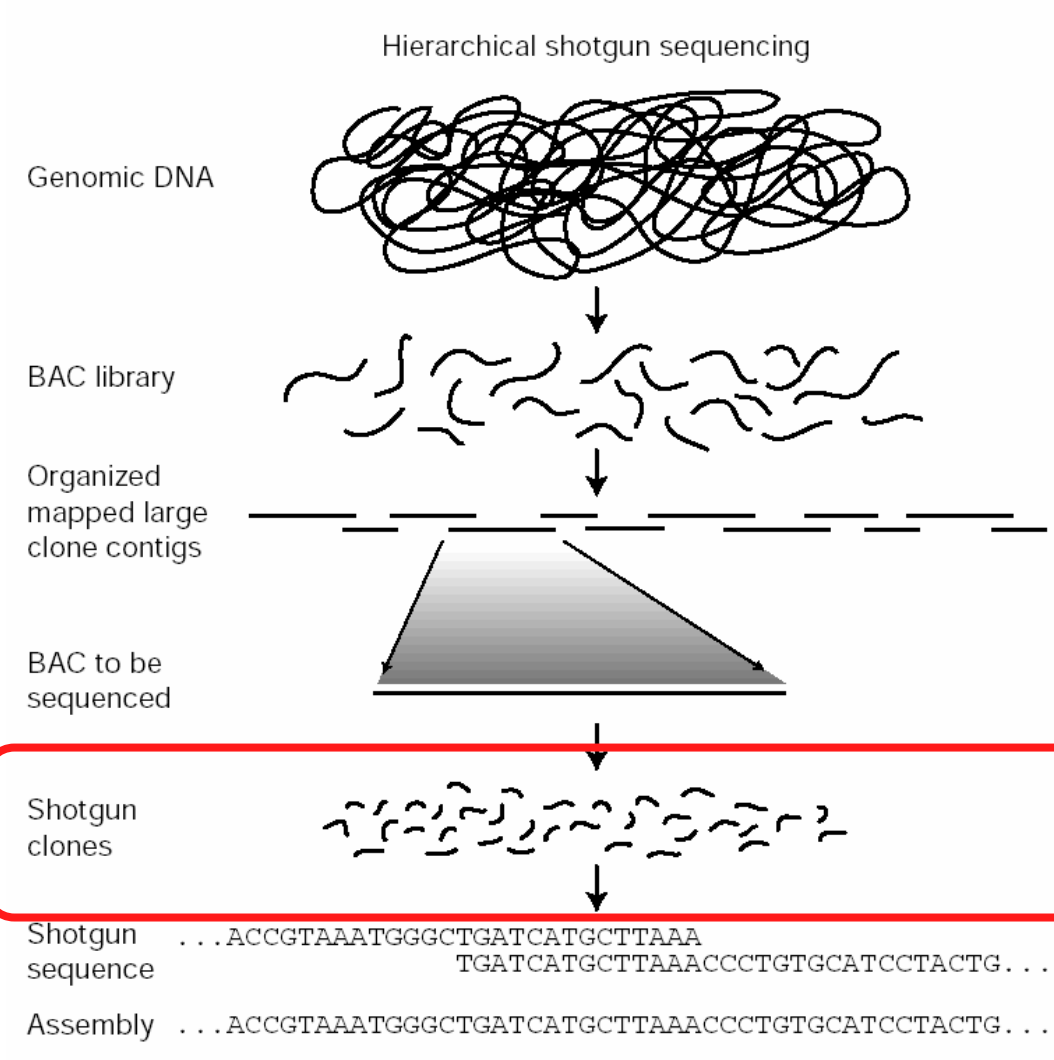




**Affymetrix GeneChip**

- **Gene Discovery Method**
  - Read out unknown chunk or genome DNA molecule from first principles, also known as **sequencing**
  - Uses clever chemical method to break down molecule and read its sequence.
  - Enzyme based methodology

# *Shotgun Sequencing Method (HGP)*



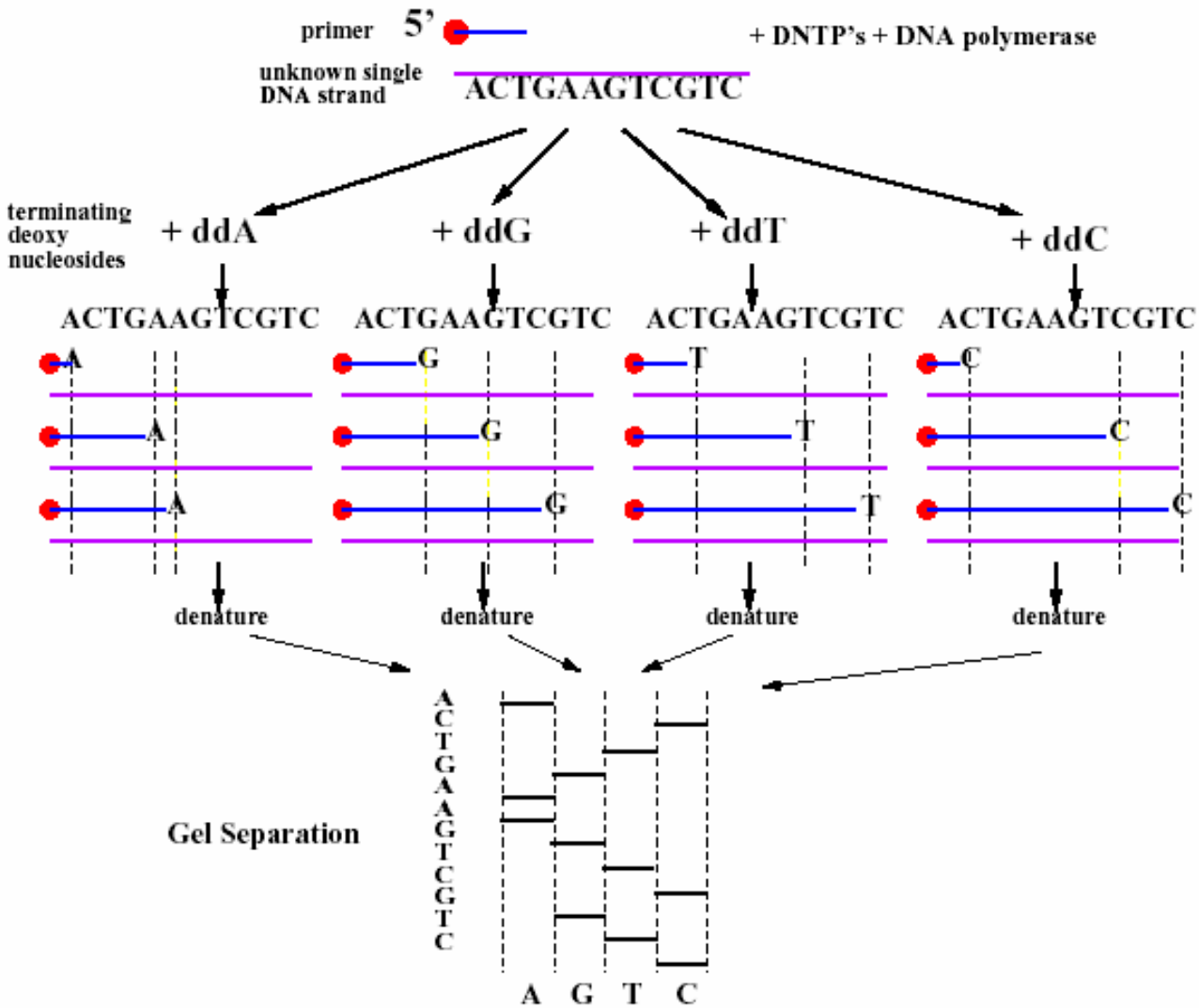

***Chromosomes (3.5 Gb)***

***Large cloned fragments (100-200 kb)***

***Small cloned fragments (500-1000 b)***

International Human Sequencing Consortium, Nature, 2001, 409:860-921

# *Sanger Ladder Sequencing Method (Circa 1975)*




***Target fragment (500-1000 b)***

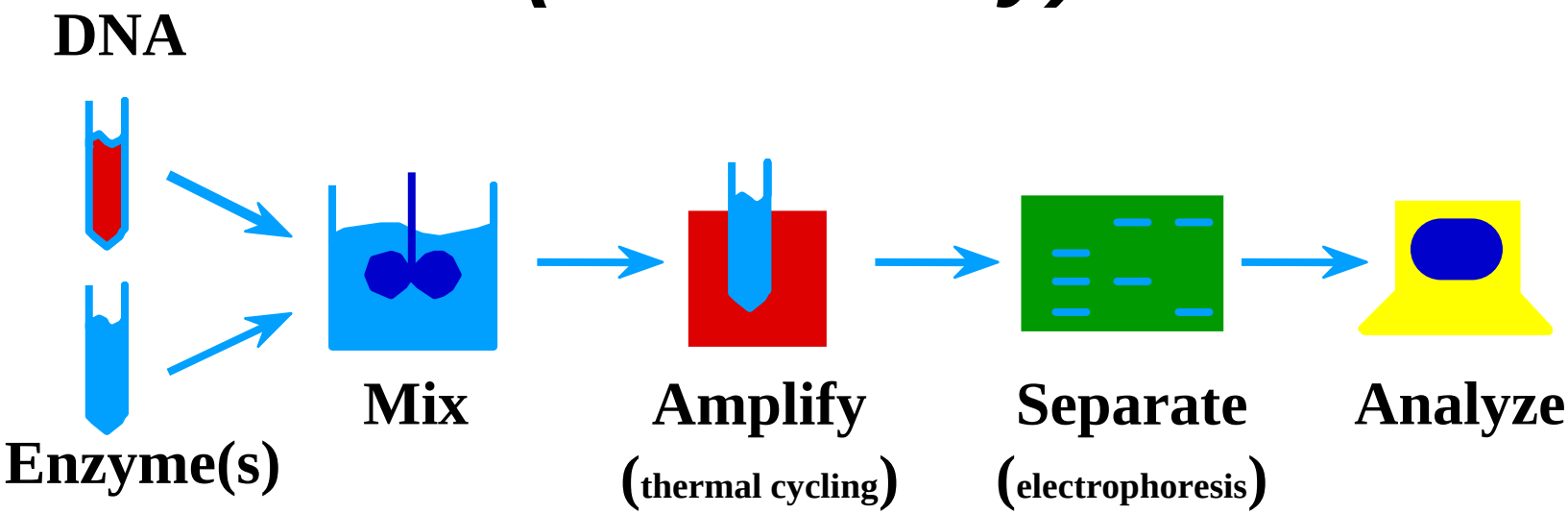
***Generation of ladder smaller fragments***

***Electrophoresis (race track for fragments under E field)***

# *Toolkit for DNA Chemical Analysis and Manipulation*

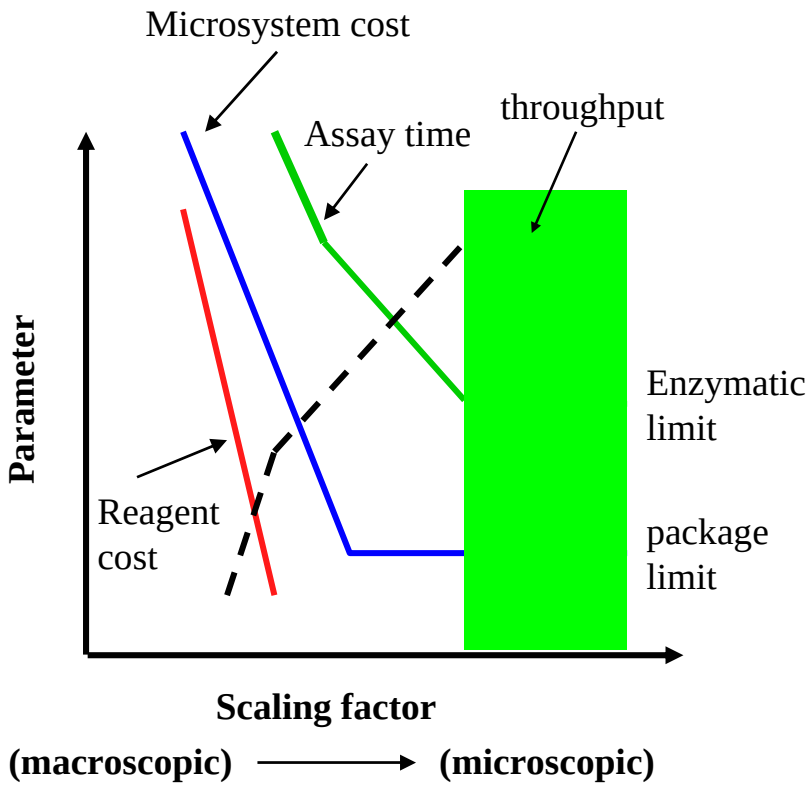
| | | |
|---|---|---|
| **DNA extraction** | Cell lysis (detergents) | Mix with detergent |
| **Chemical amplification** | Increase concentration of flanked DNA fragment | PCR/SDA mix and thermal cycling |
| **Digestion** | Cut DNA fragments in small pieces (enzymes) | Mix with enzyme |
| **Separation** | Fragment separation due to size/mobility difference | Electrophoresis (race track for DNA fragments) |
| **Sequencing** | Read out polymer sequence | Sanger reactions (randomly terminated amplification)<br>+ separation |
| **Hybridization** | Detection of formation of double stranded DNA | Mix with (immobilized) target probe |
| **Labeling and staining** | Fluorescent or electrochemical tagging | Mix with binding tag<br>Observe response (detection) |

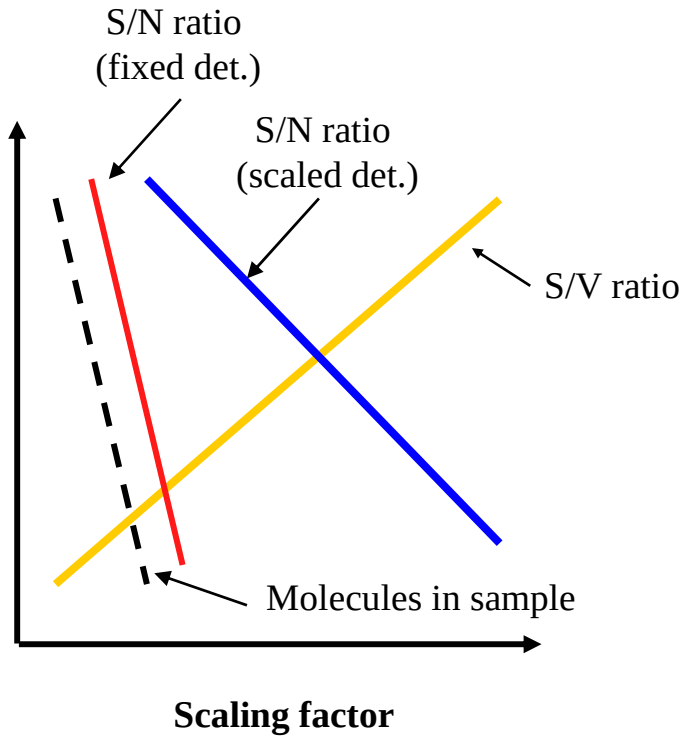
# *Traditional Chemical Analysis (DNA Assay)*




- Current sequencing assays are fairly expensive due to equipment and labor costs and requires a lab
- Lots of outsourcing available though !
- Presently $25MM/genome
- Would like $100K/genome → $1000 genome (NIH mission)

# Benefits of Miniaturization and Scaling

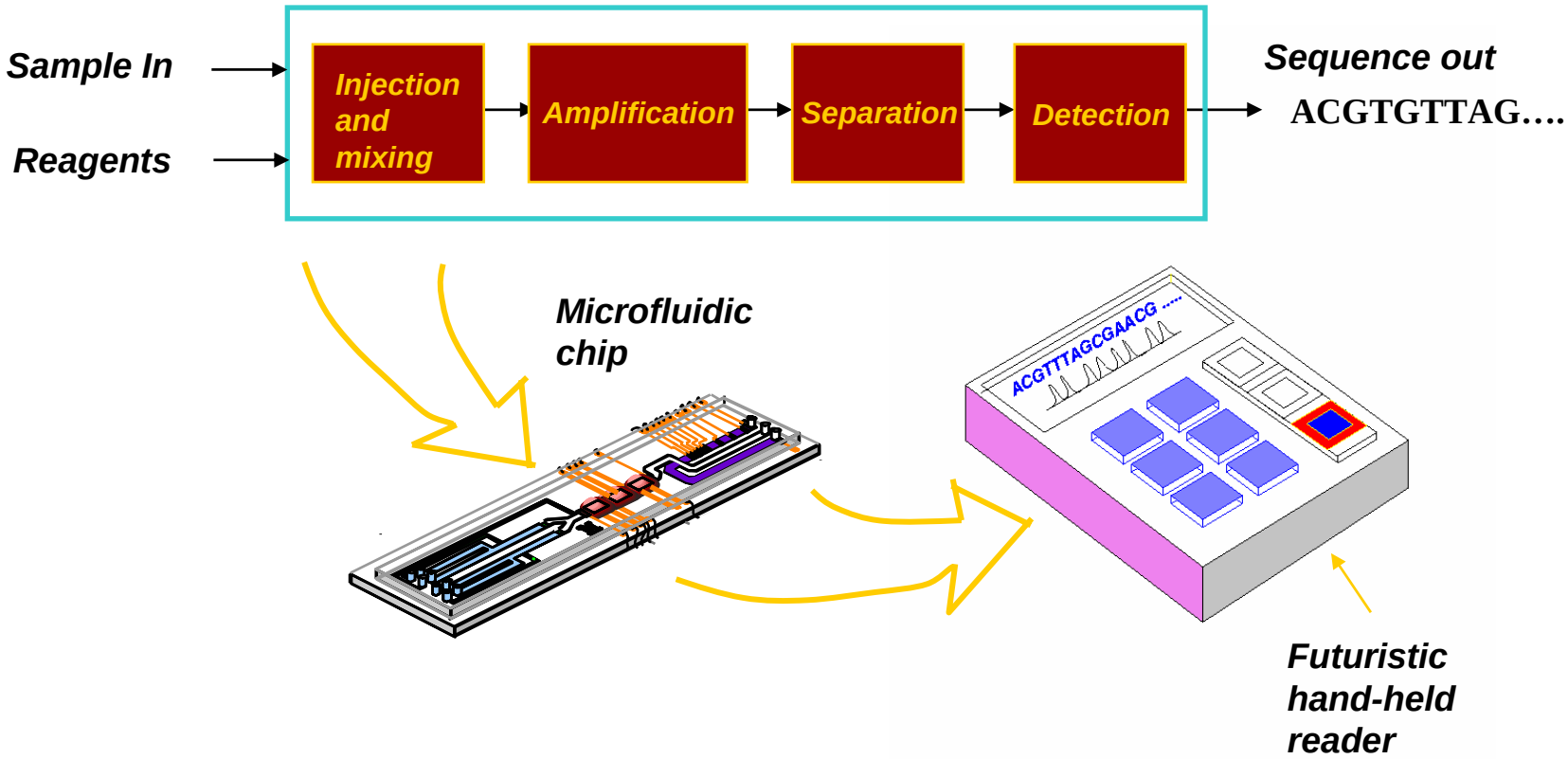


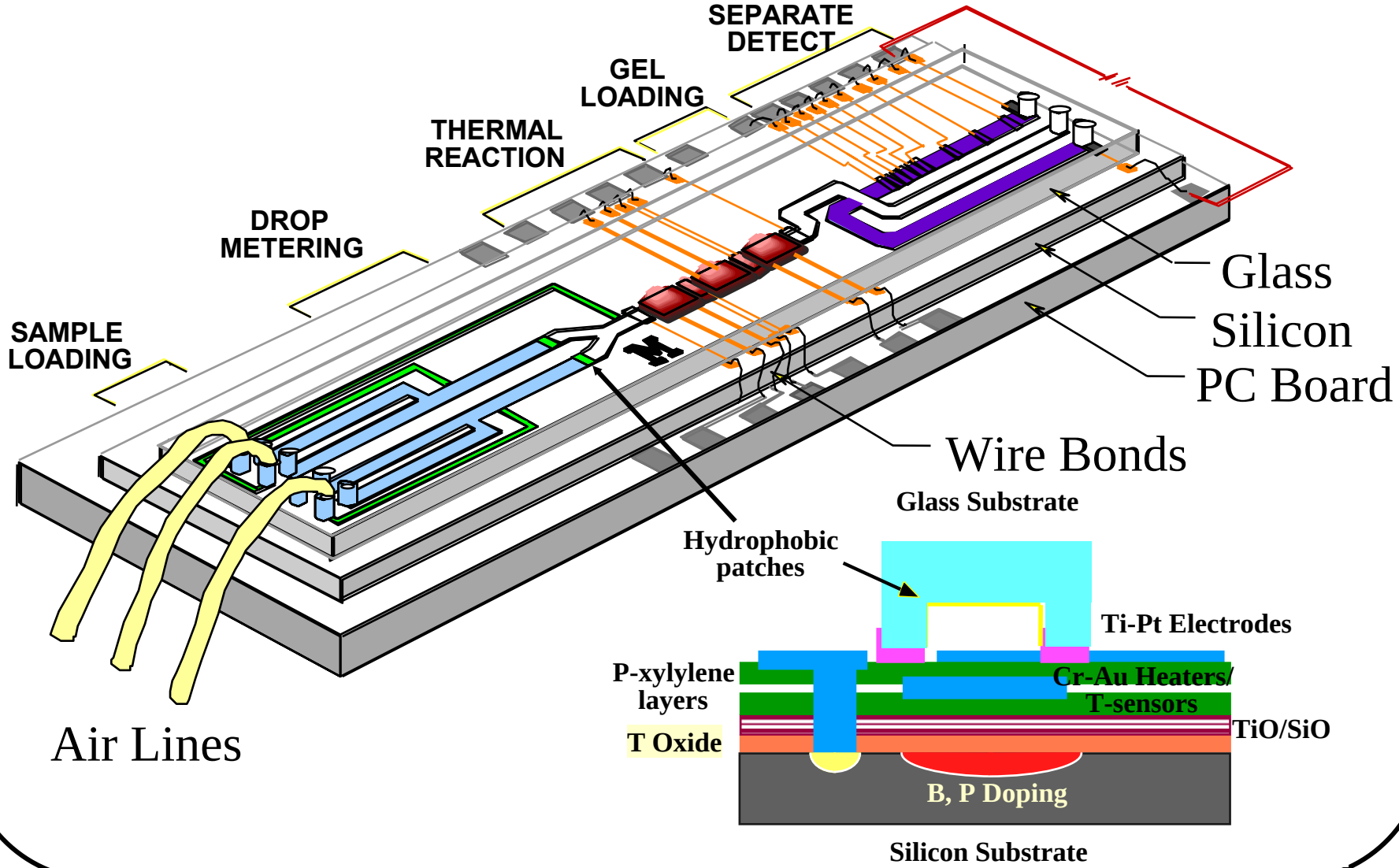

- Miniaturization reduces cost and increases throughput
- But, reduced S/N ratio and increased S/V ratio are challenges

# The Need for Integration

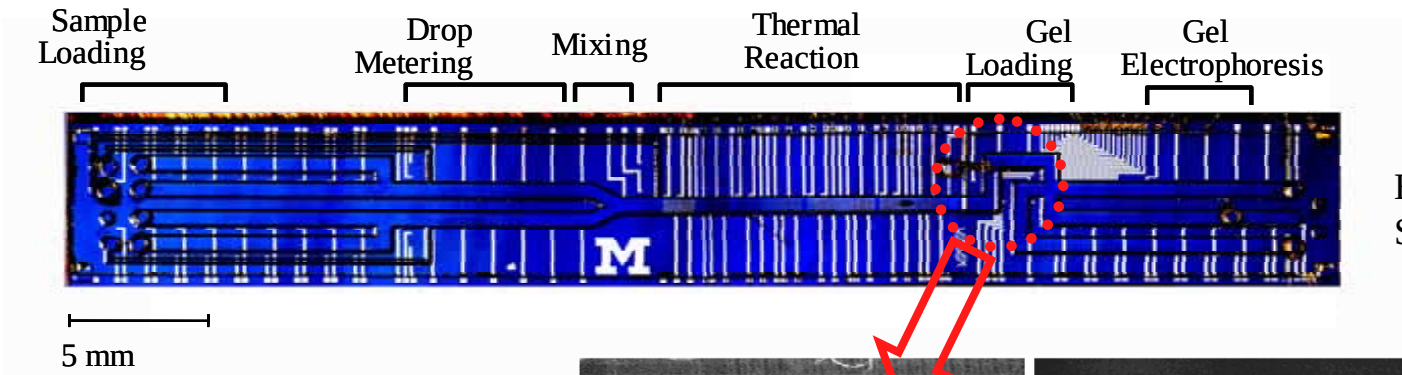
- ***Sample-to-sequence device is highly desirable (eliminates all manual transfers and interactions)***



# Integrated DNA Analysis Chip

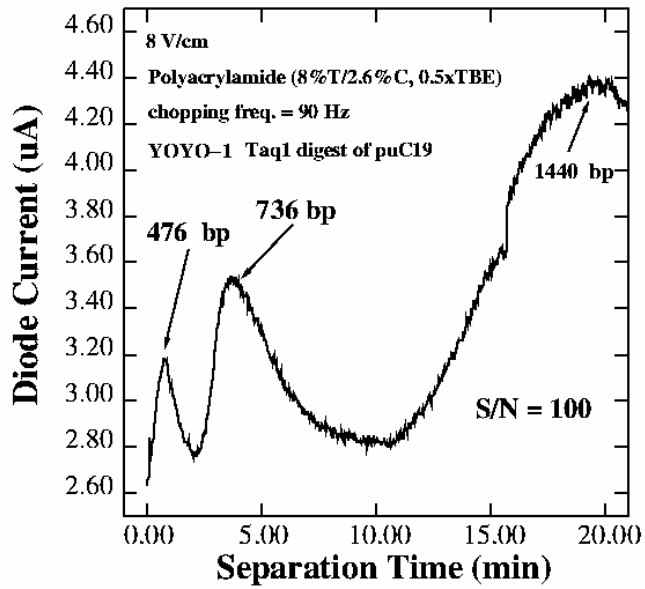


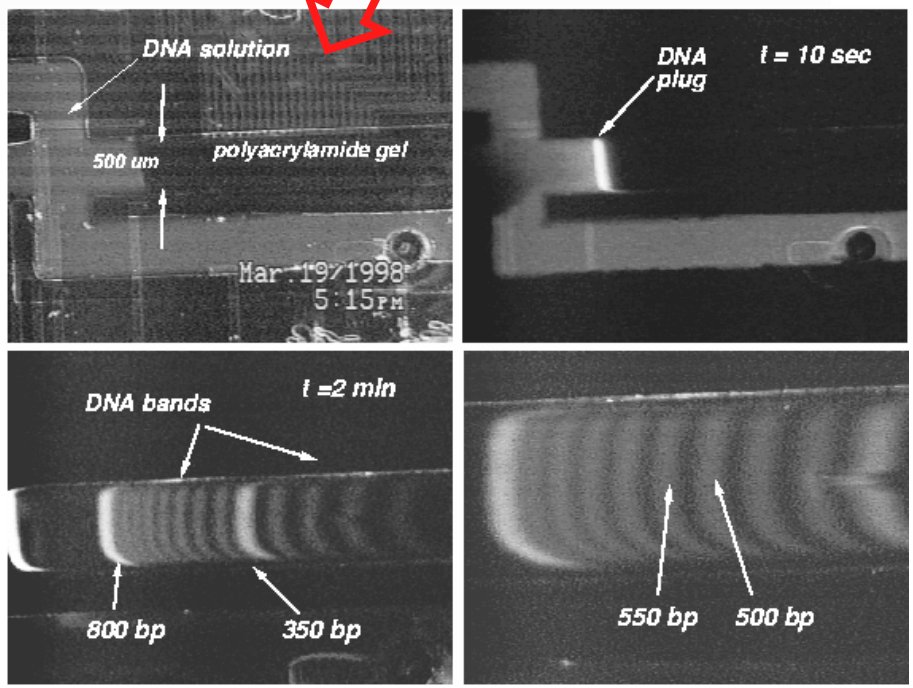
# *Si-Glass autonomous microchip with amplification, separation and detection functions*




Burns Science 98

- **Individual Drops (~100 nl)**
- **Integrated Heating (± 0.1 C)**



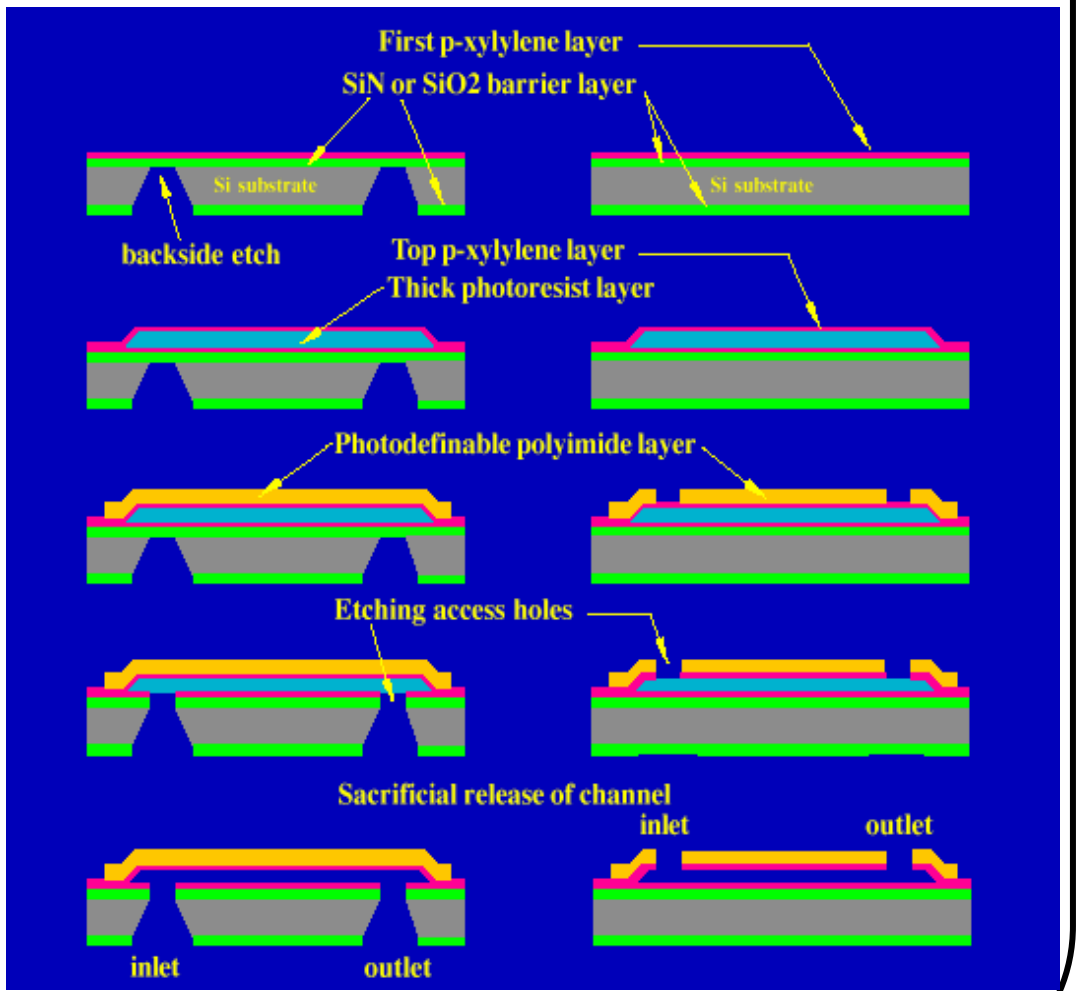




- **Integrated Detection MDS (10 ng/µl)**

# *Microfluidic Technology using Low-Temperature Surface Micromachining of Parylene Films*

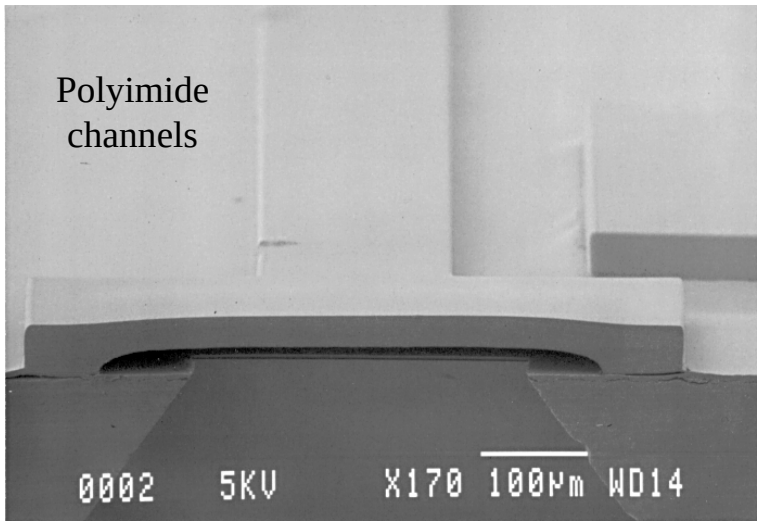
# *Surface Micromachined Plastic Channels*

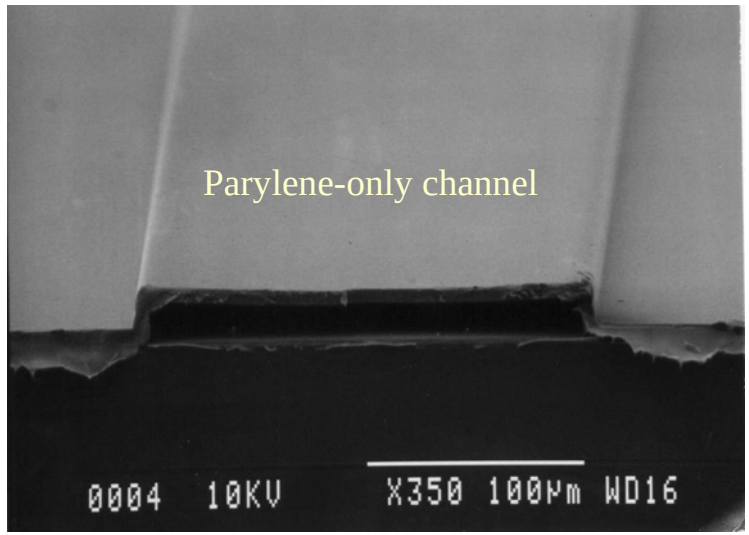
- Process uses thin plastic films deposited onto a silicon or less expensive plastic substrate
  - Uses sacrificial tech.
  - 20-80 µm-high structures
  - Transparent
  - Biocompatible

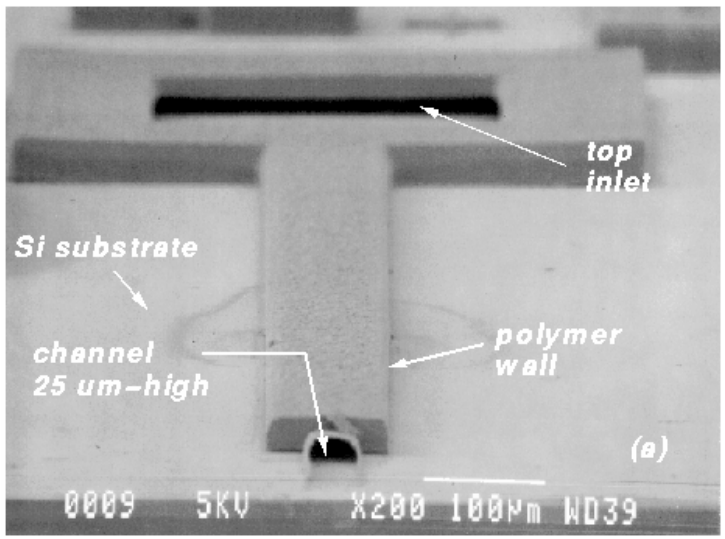


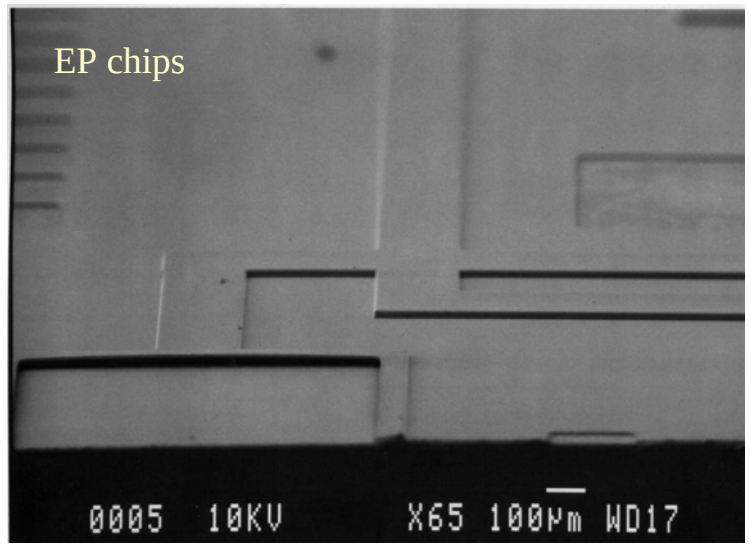

Man, MEMS 97

# Plastic Microfluidic Structures

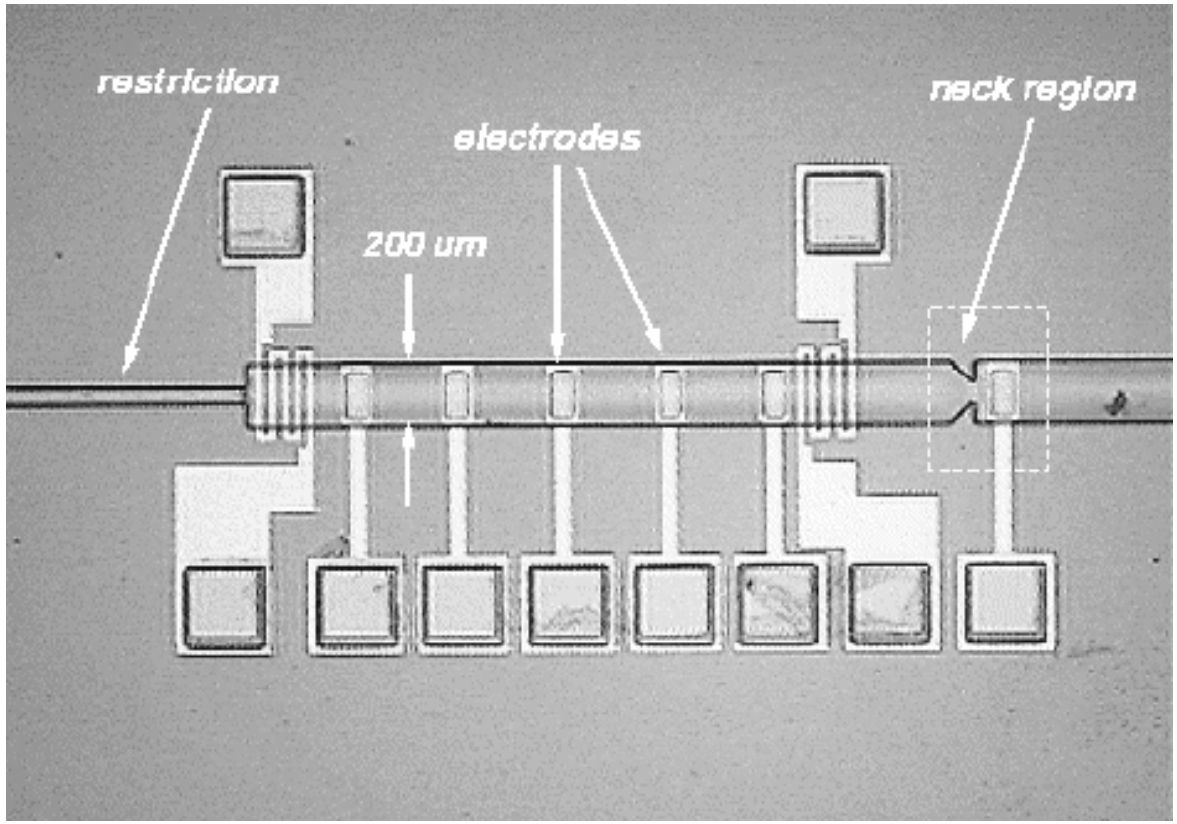


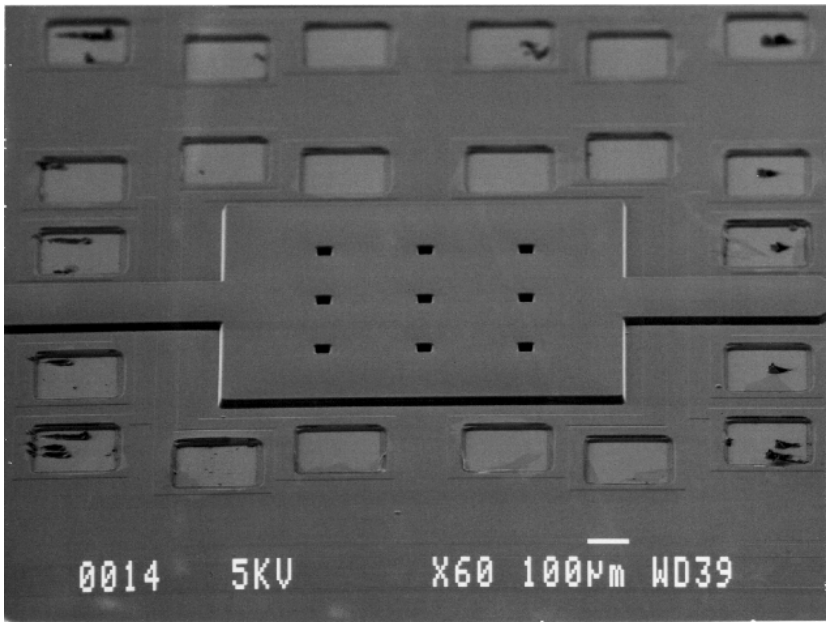



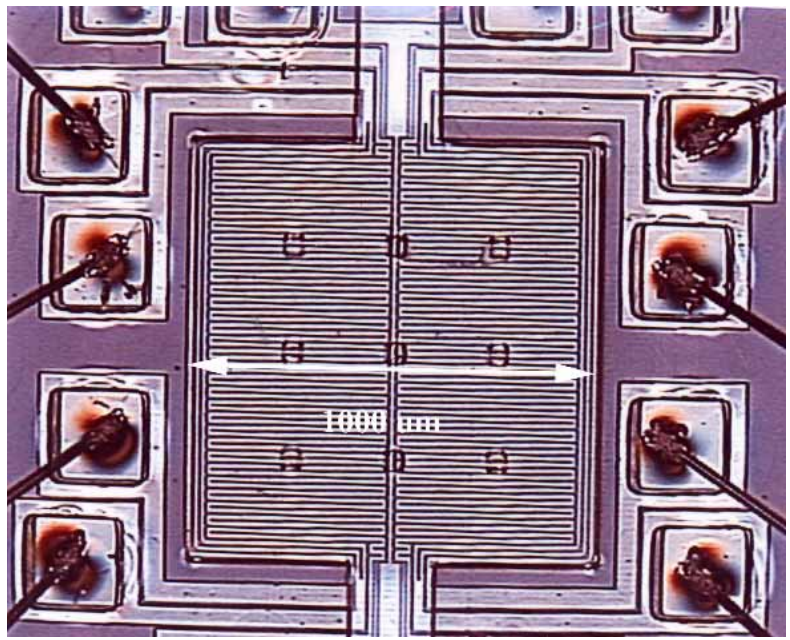
# *Multilevel Metals on Plastics*




- Ti/Pt and Ti/Au trapped between plastic layers

# *Thermally Isolated Structures*

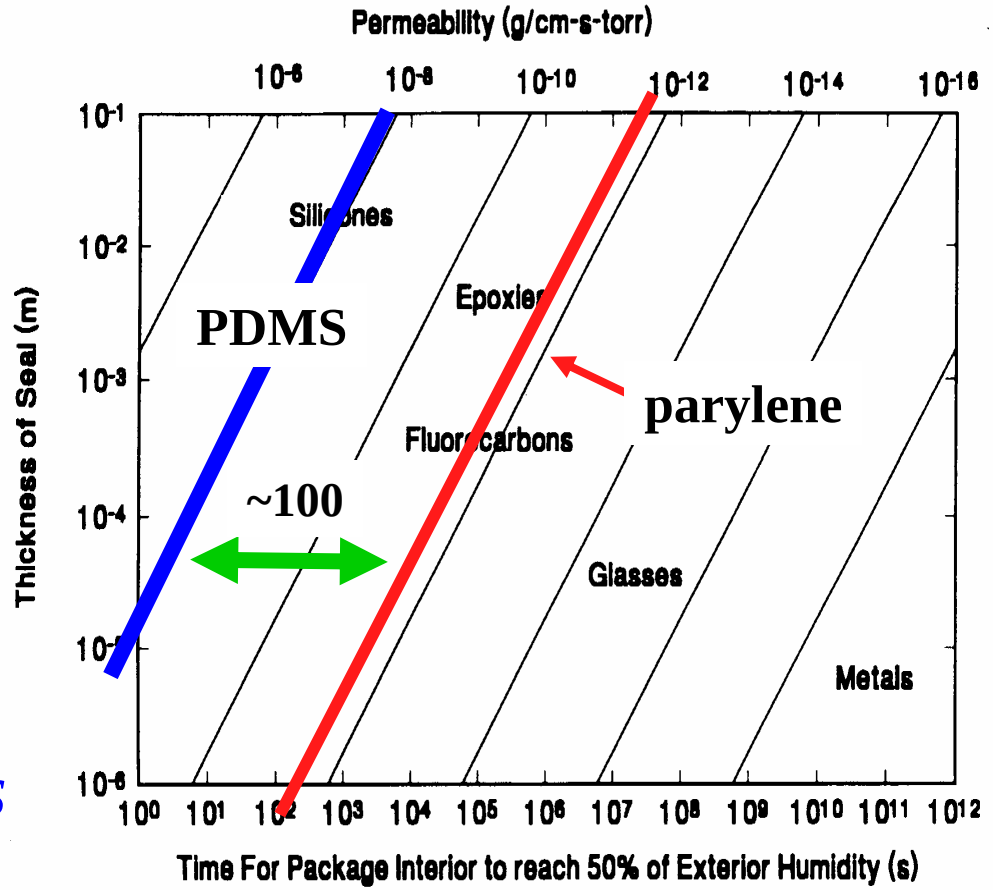


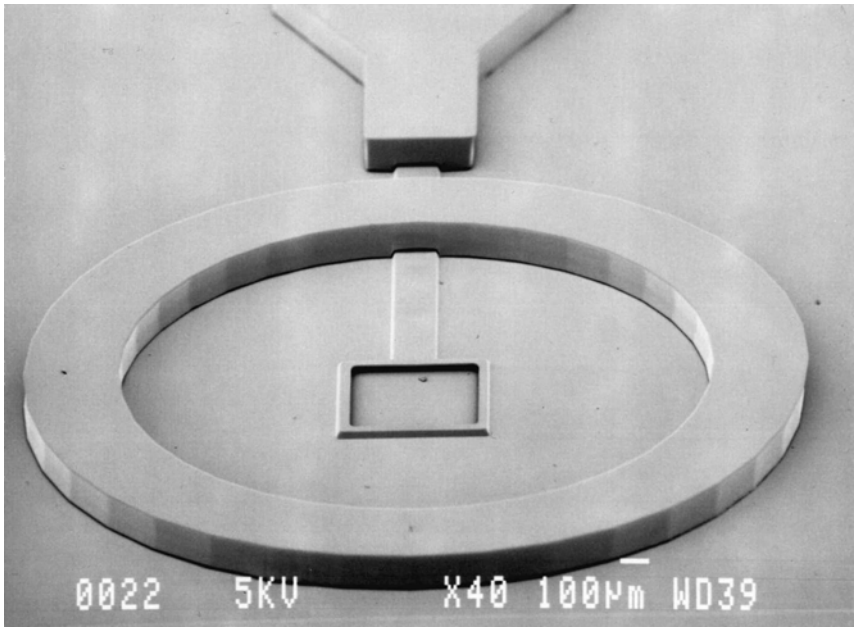

- Use thick plastic layer below heater

# *Permeability In Plastics*

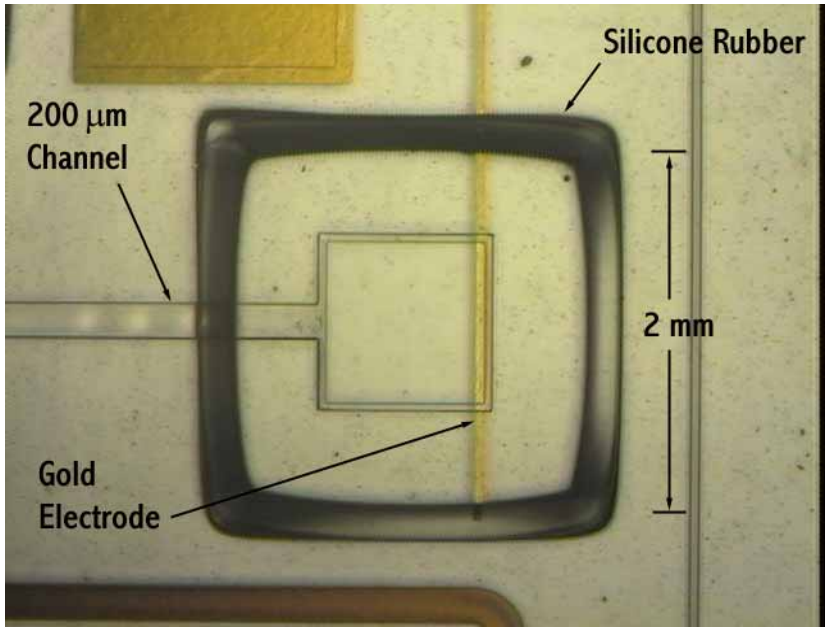
- Gas diffusion in plastics is high
- Permeability coefficient is high
  - $J = ( D/t ) P_v / kT$
  - $\Rightarrow$ Evaporation of sample through walls is fairly rapid
- *May needs thick walls or diffusion barrier*



# *Thick Walled Structures*



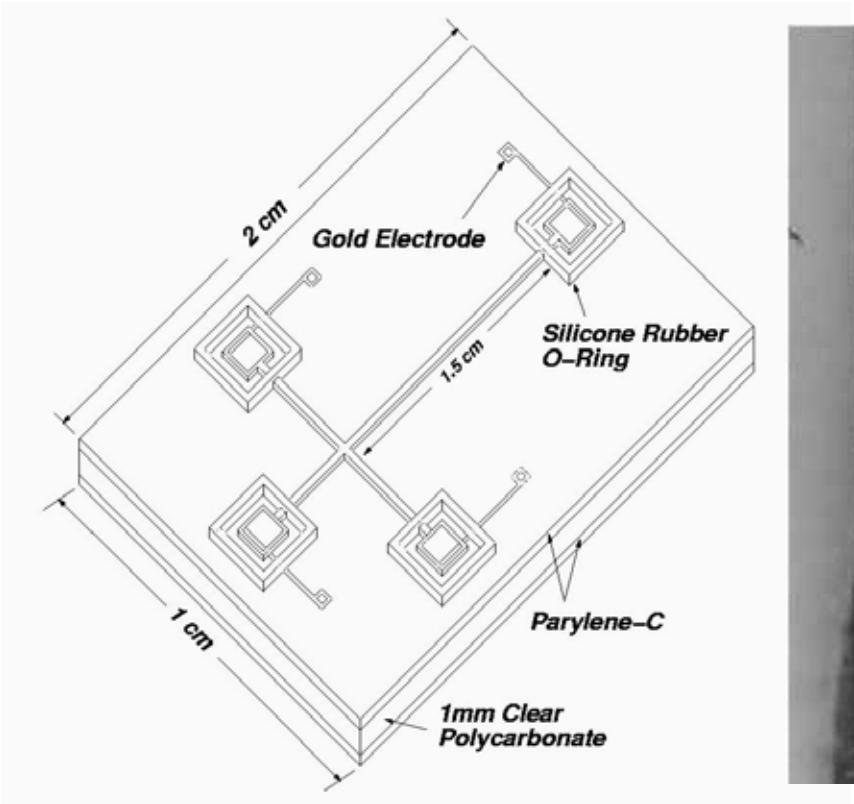

**200 μm-thick EPON wall**

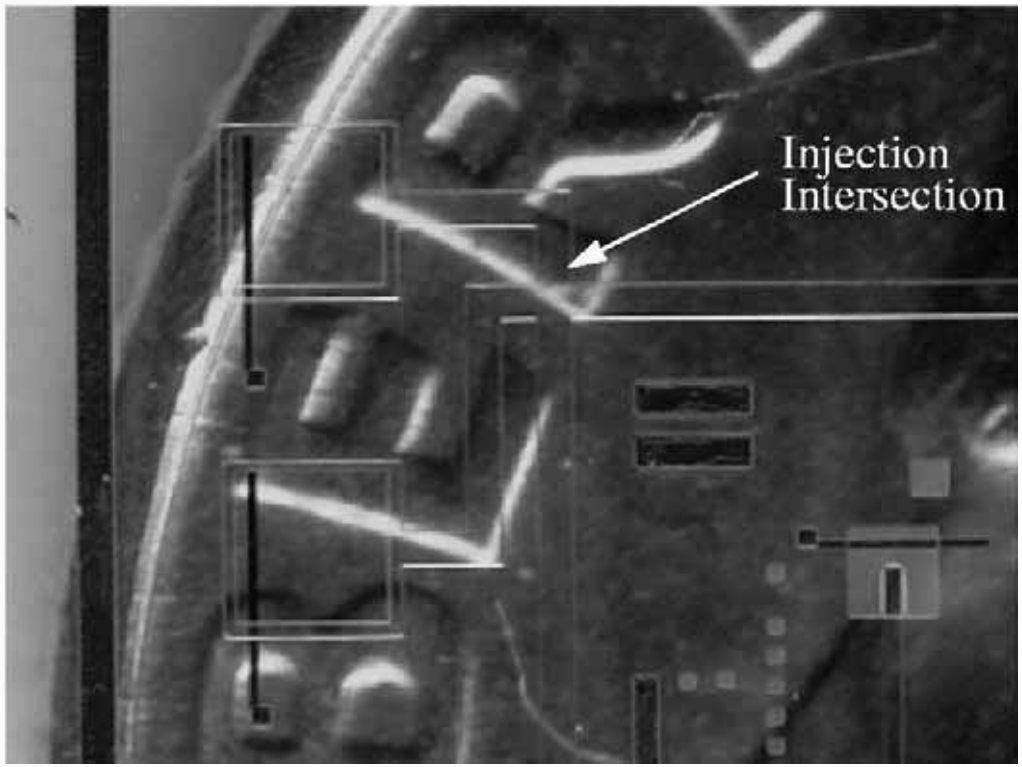



**500 μm Photopolymerized silicone rubber**

- EPON resist or Photopolymerized silicone rubber

# *Passive Plastic Electrophoresis Device*



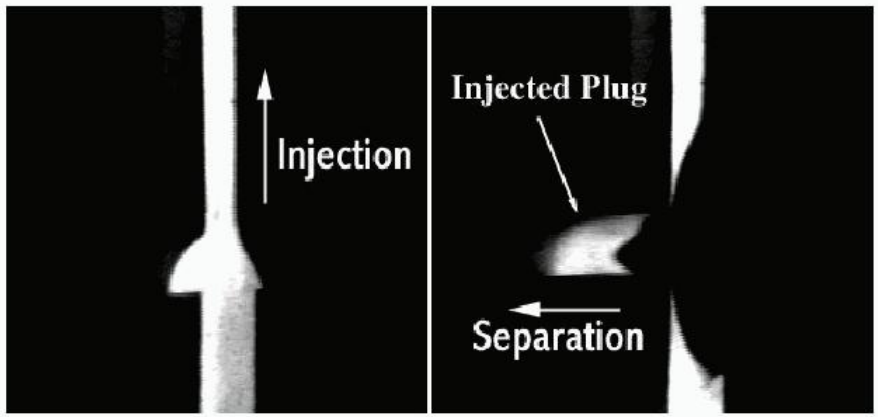



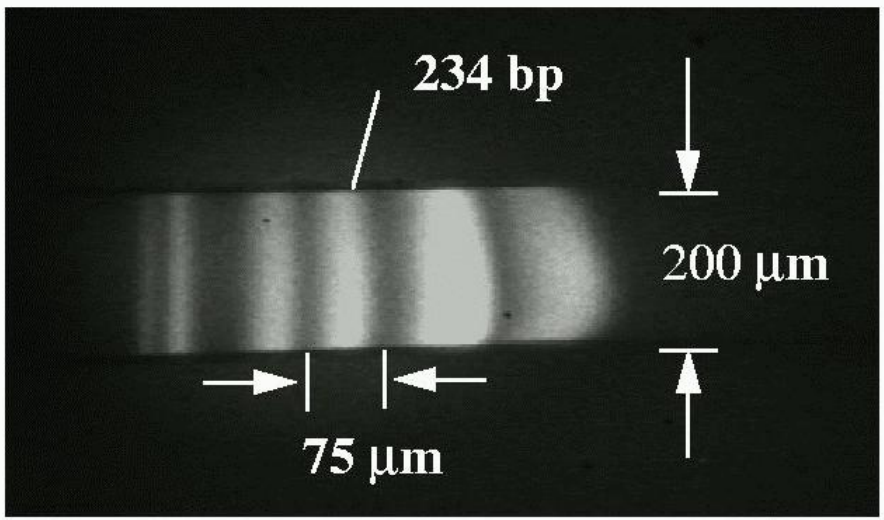

- ***Polycarbonate wafers are inexpensive and transparent***

Webster
MicroTas 98

# *DNA Sample Injection*

**Injected Plug ~300 μm Wide**




**10 minute Pre-Electrophoresis**

**234 bp Band compacted 4 times**

# *DNA Ladder Separation*

**HaeIII digest of ΦX174 RF DNA**

**0.5% HEC**

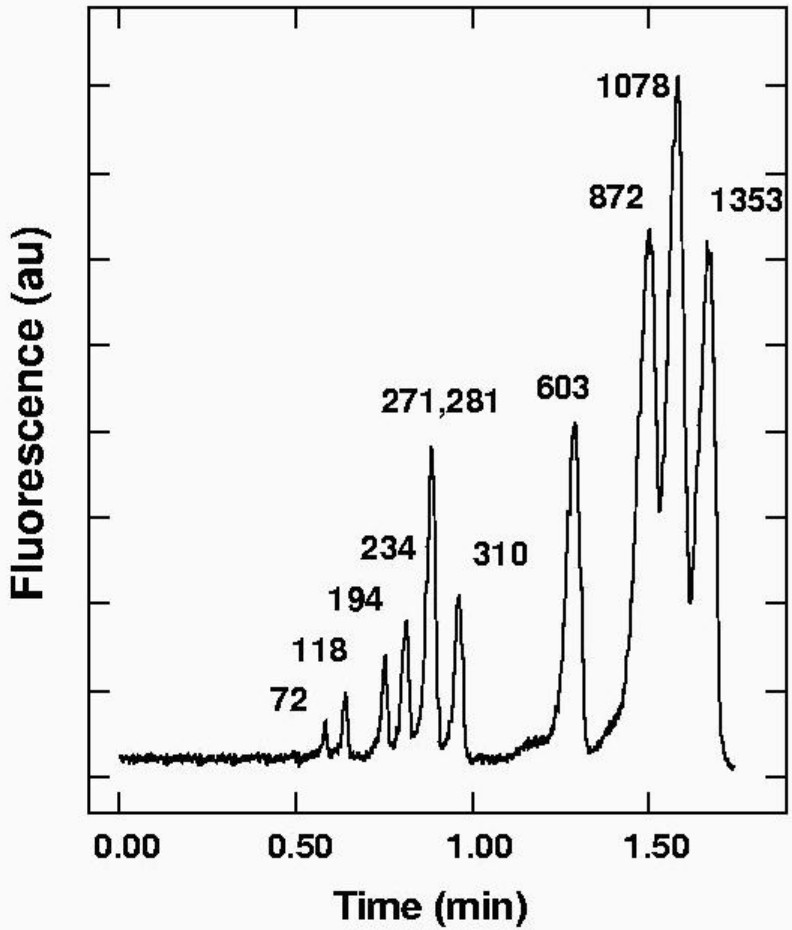
**0.1xTBE**

**110 V/cm**

**SYBR Green I Dye**

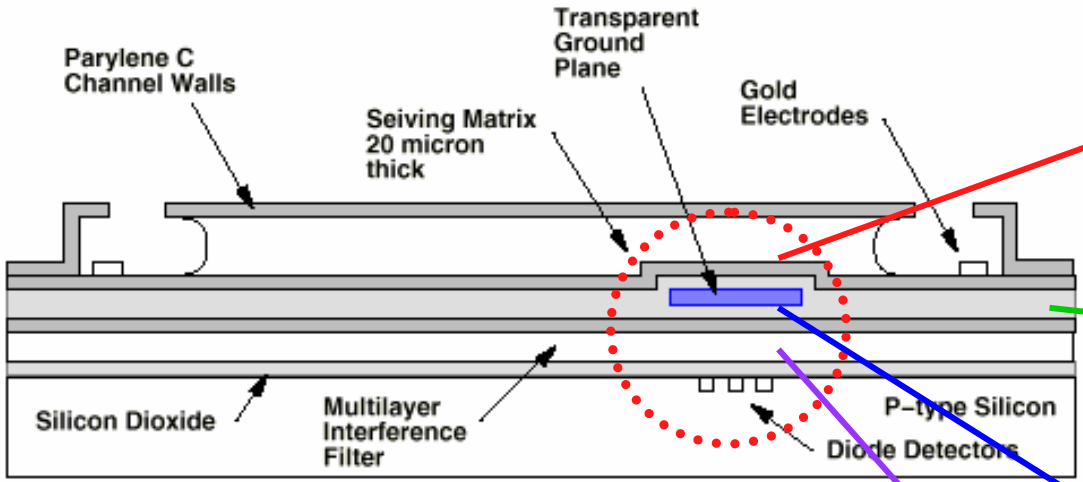
**1.5 cm Separation length**

**234 bp ~100000 Theoretical Plates**

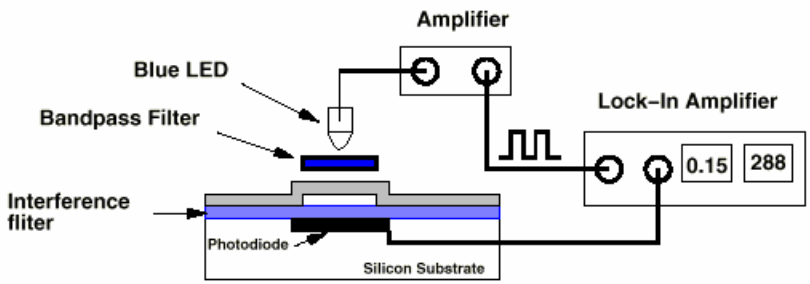



**Theoretical Plates = (maximum number of resolvable bands)$^2$**

# *Electrophoresis Chip With Integrated Fluorescence Detector*



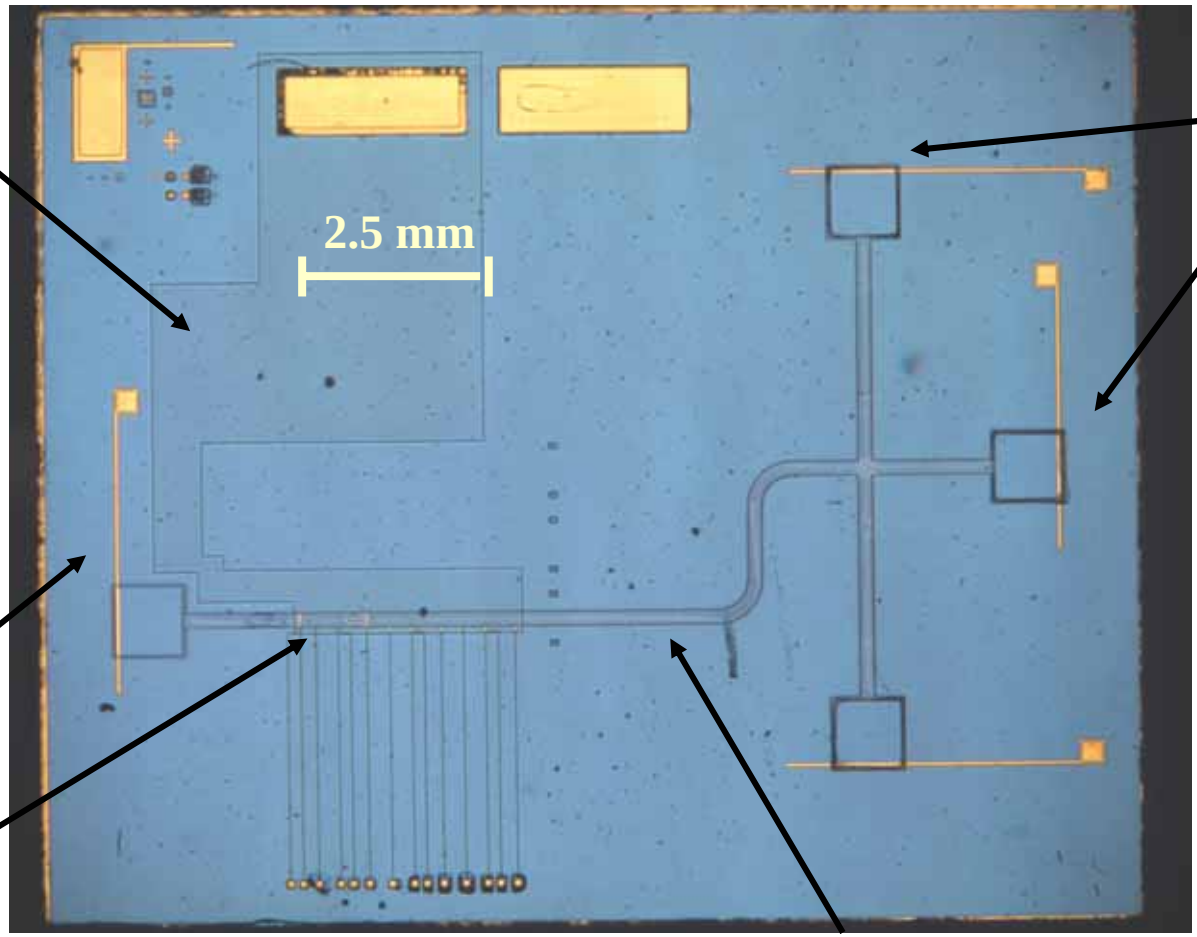

**On-chip fluorescence detection system**




## *Four Levels of Isolation*

- **Chemical Isolation**
  - of sample from diode (diffusion barrier for buffer salts)
- **Dielectric Isolation**
  - from high voltages in EP channel (thick dielectric)
- **Field Isolation**
  - from channel field effects (transparent AZO ground plane)
- **Optical Isolation**
  - of excitation light (interference optical filter)

# *Electrophoresis Chip With Integrated Fluorescence Detector*




Webster
MEMS 2000

# Monolithic Structures for Integrated Electrophoresis Systems

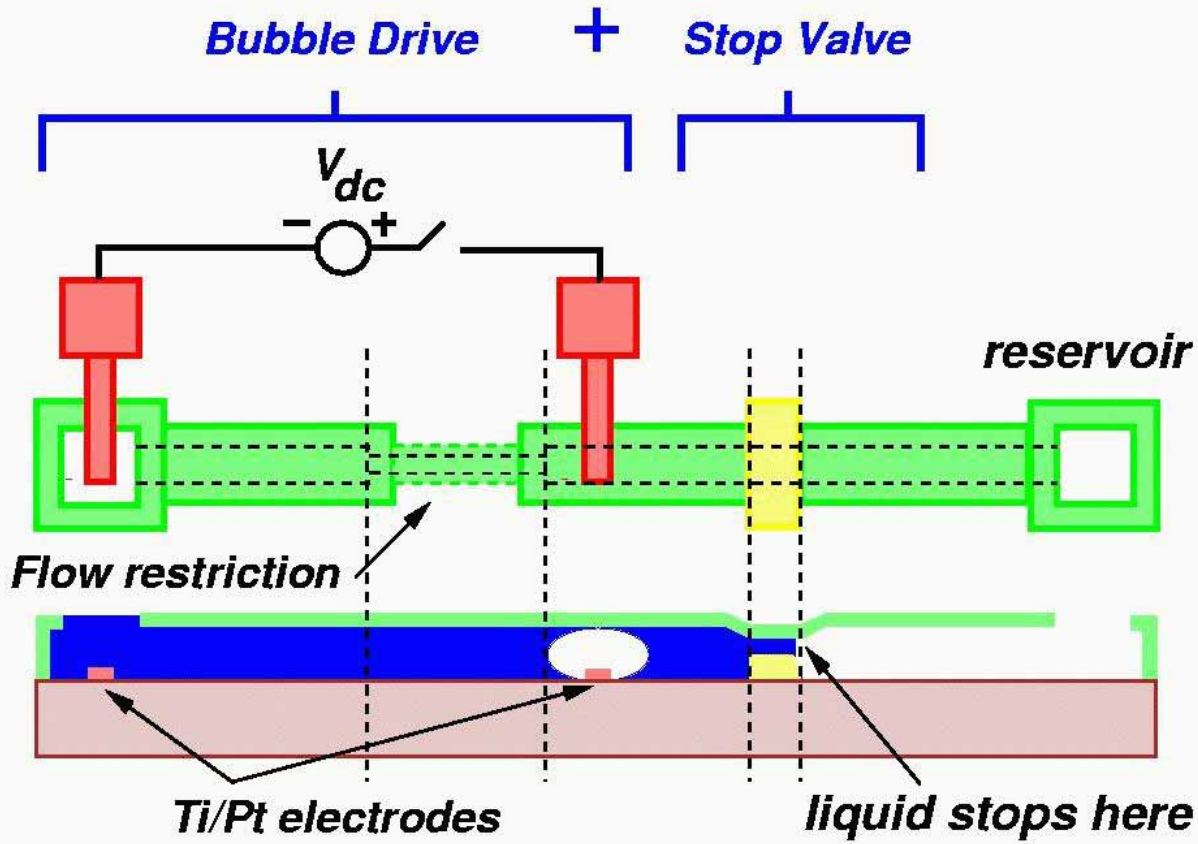
by

James R. Webster

## DNA Separations on Microchips

*Injectors, Pumps, and Actuators Integrated with Surface Micromachining of Parylene Films*

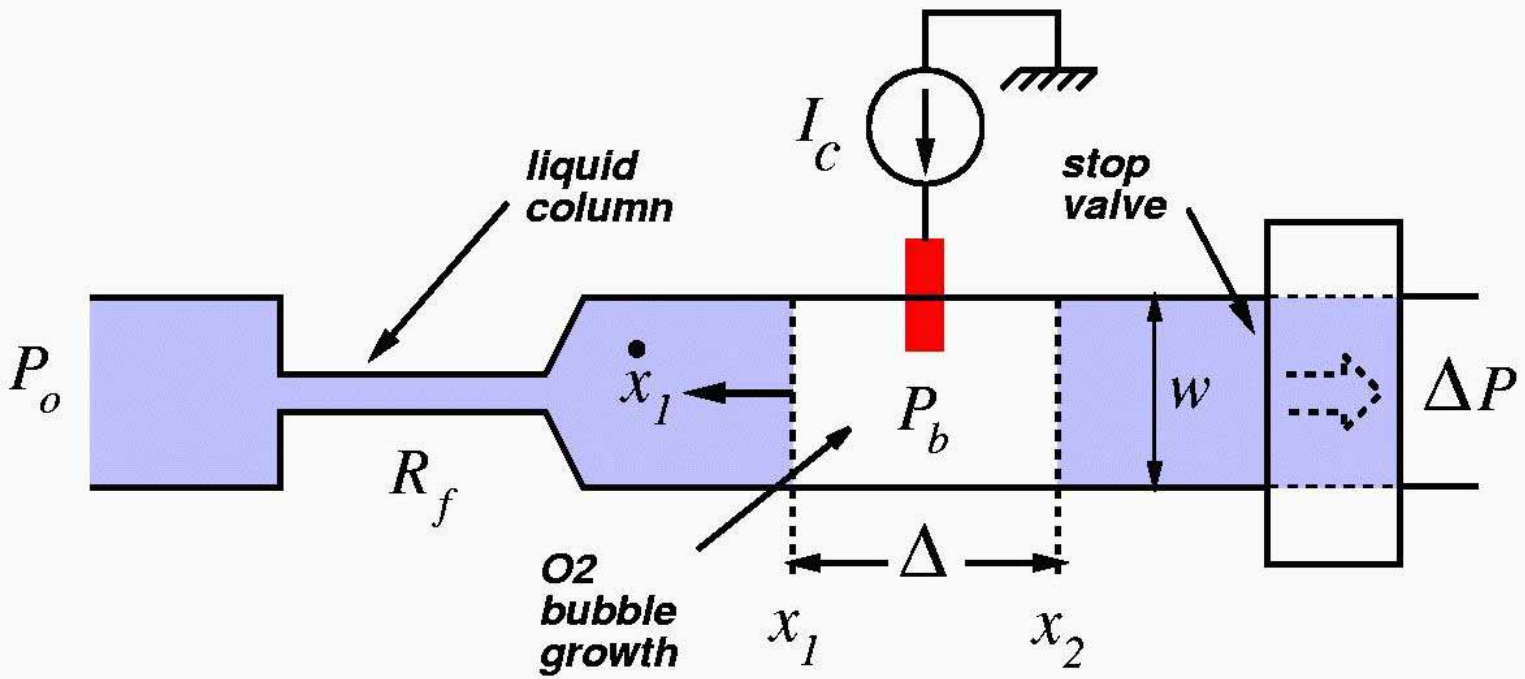
# Capillarity Driven Injector




- Propulsion mechanism --- electrolysis
- Stop valves ( vertical and horizontal )

P. F. Man
MEMS 98

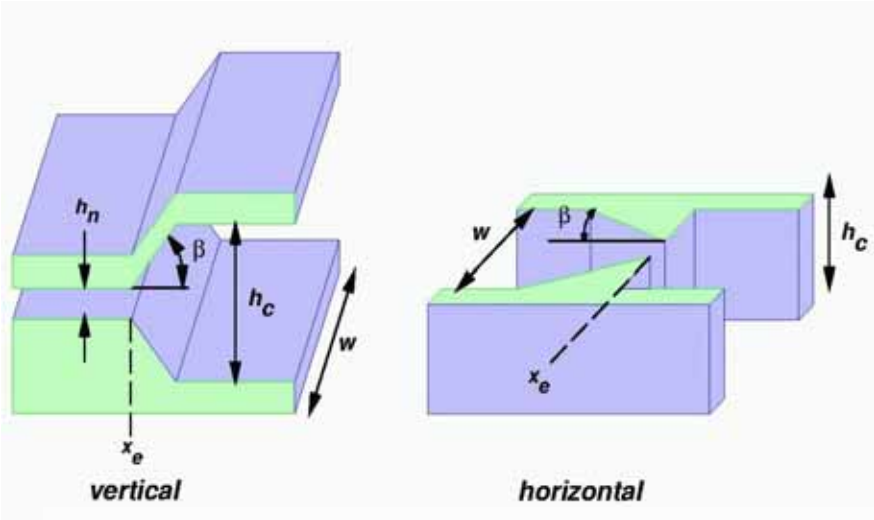
# Principle of Injector



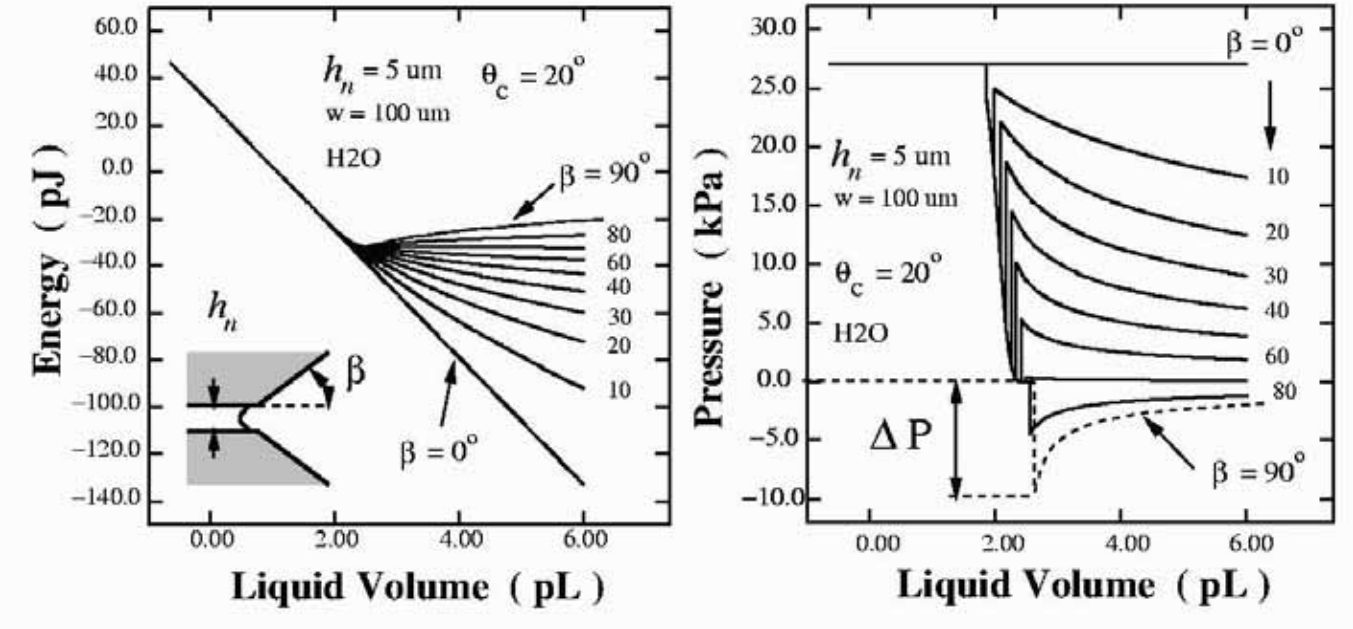

- **Threshold current**
- **Typically 5 - 10 uA**

$$I_c = \frac{\Delta P^2}{R_f m k_B T}$$

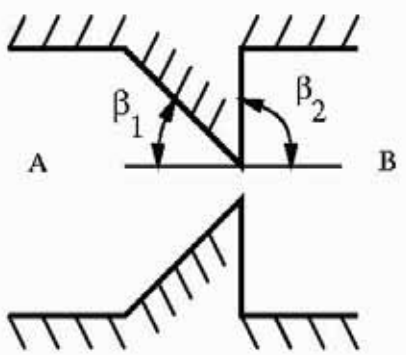
# Types of Neck Valves

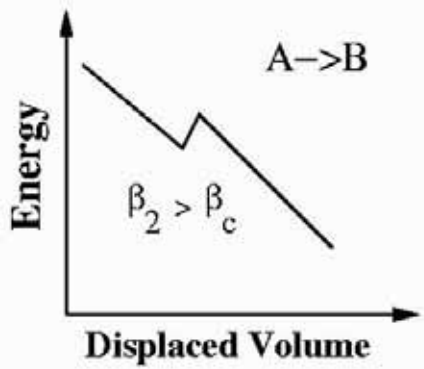


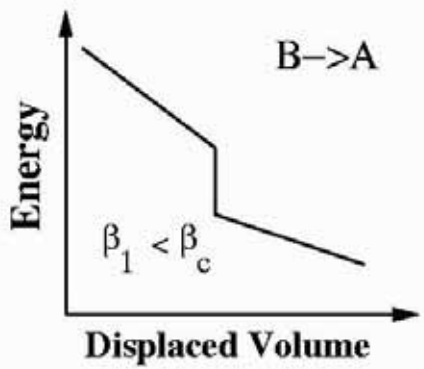

- Discontinuity of wall slope can create energy well and pressure barrier that stop wicking
- Wicking resumes if external pressure overcomes barrier

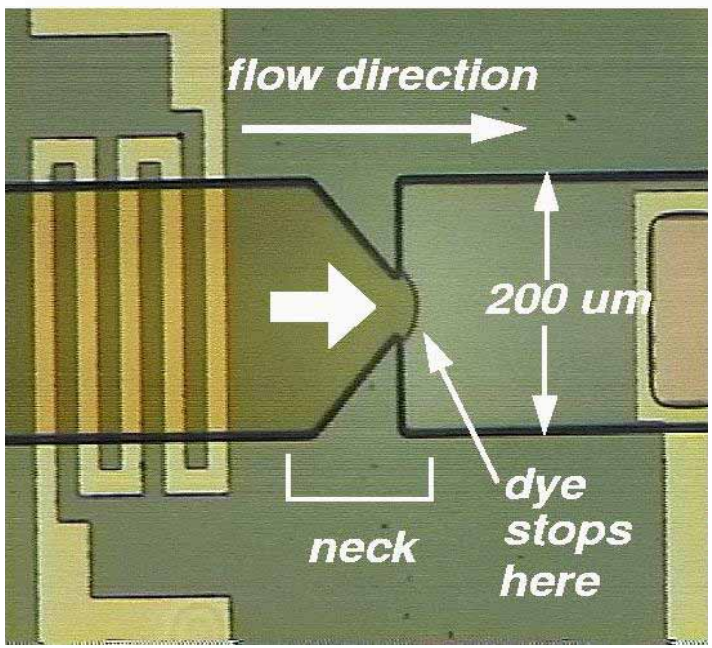


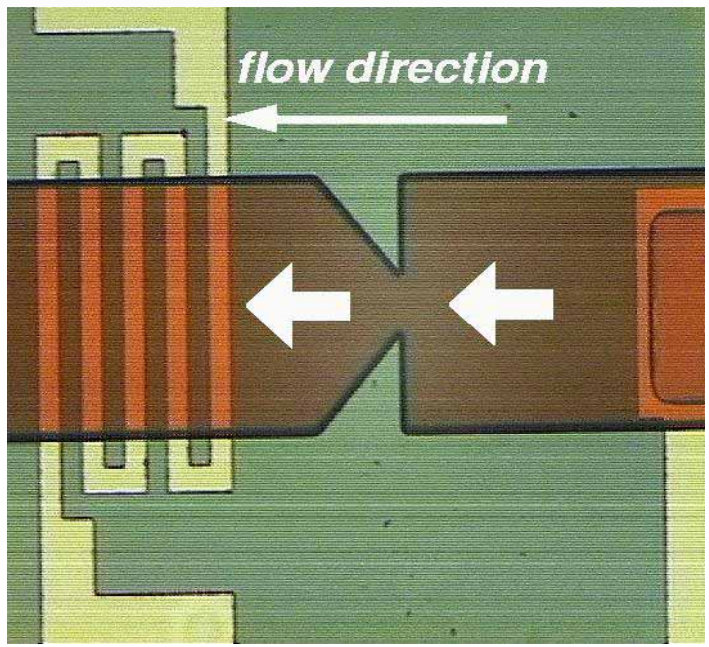
# Unidirectional Stop Valve



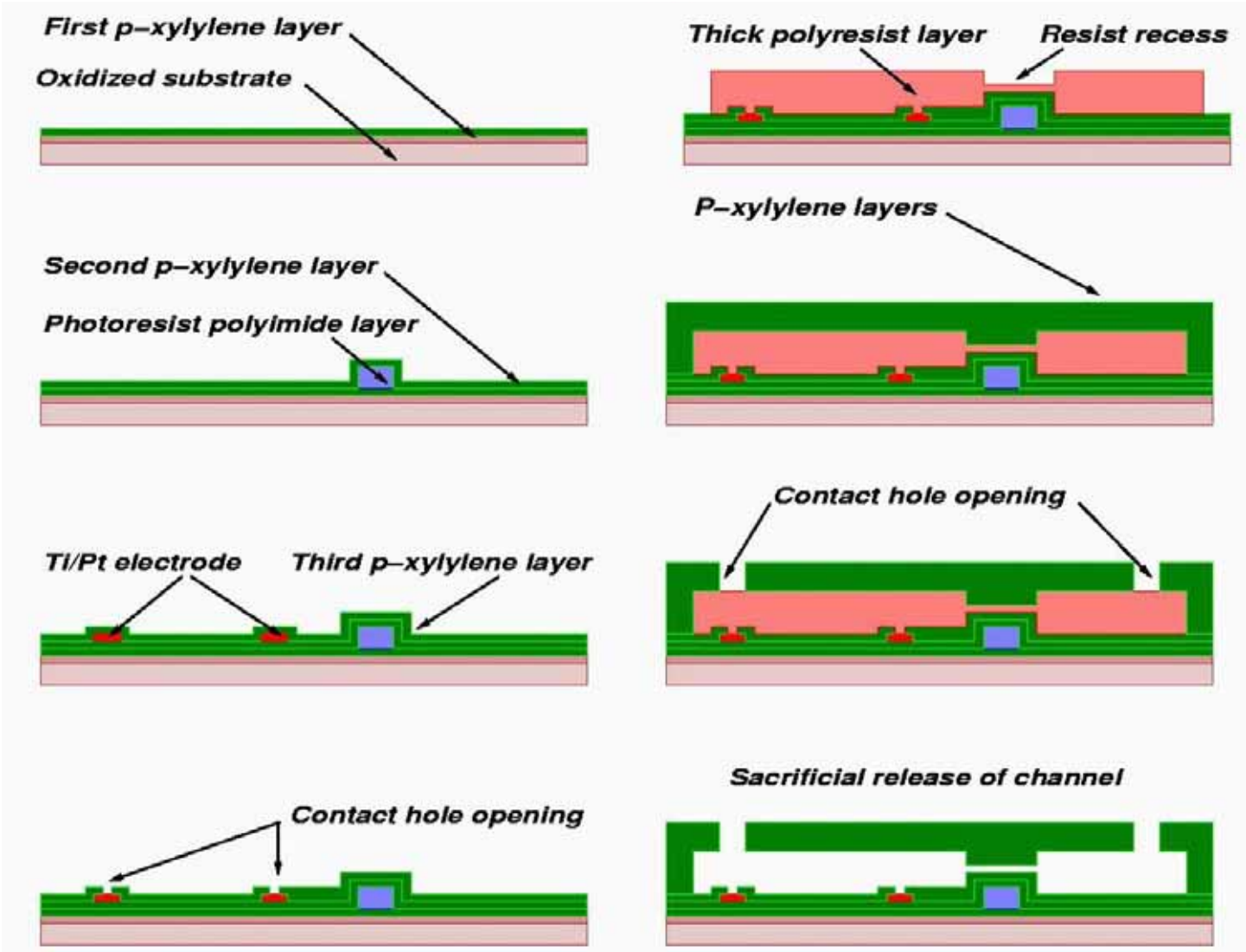



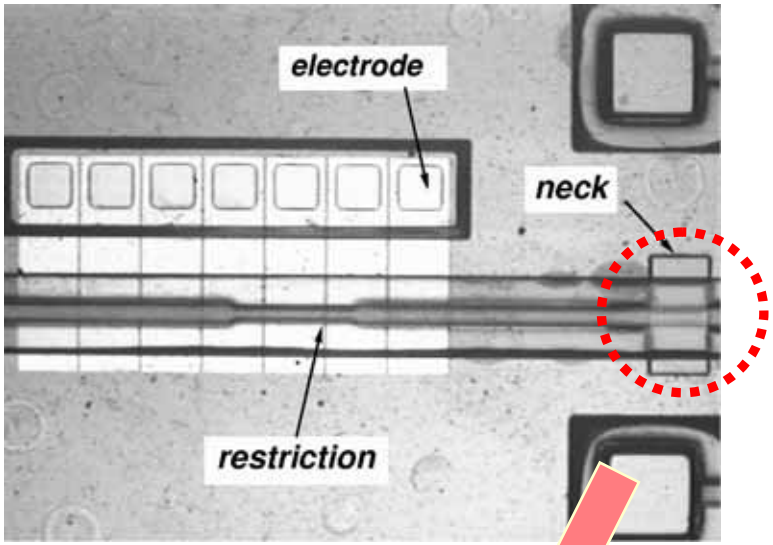



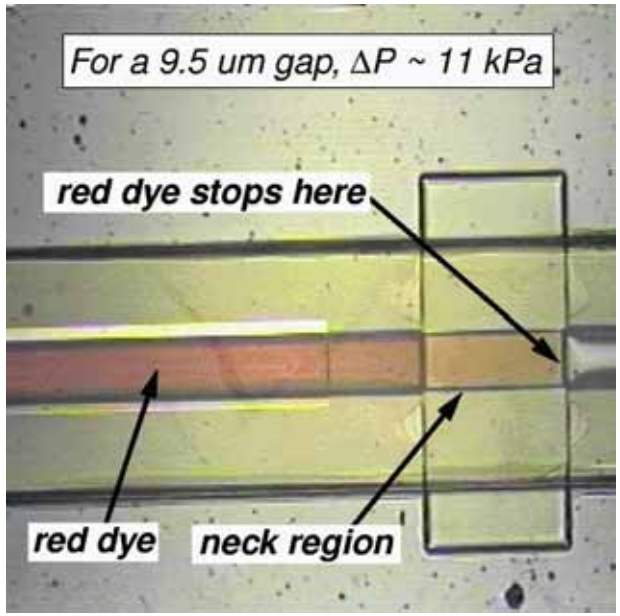



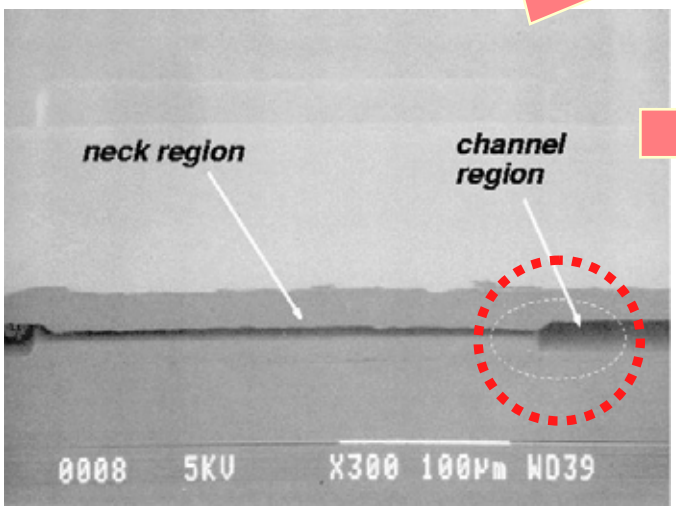
# Fabrication Process

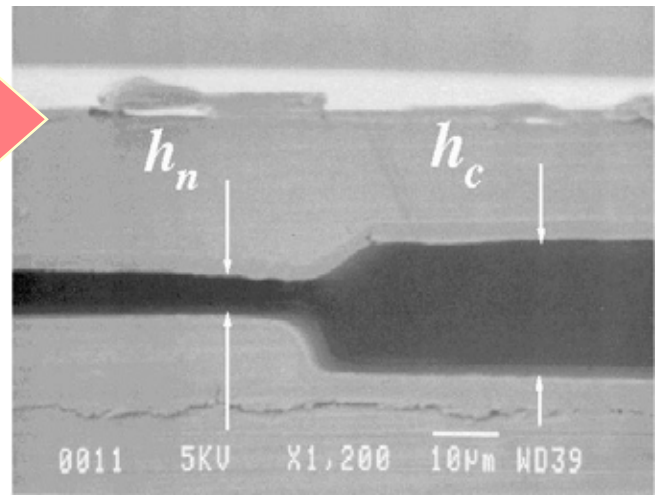


# Plastic Injector with Vertical Stop Valve

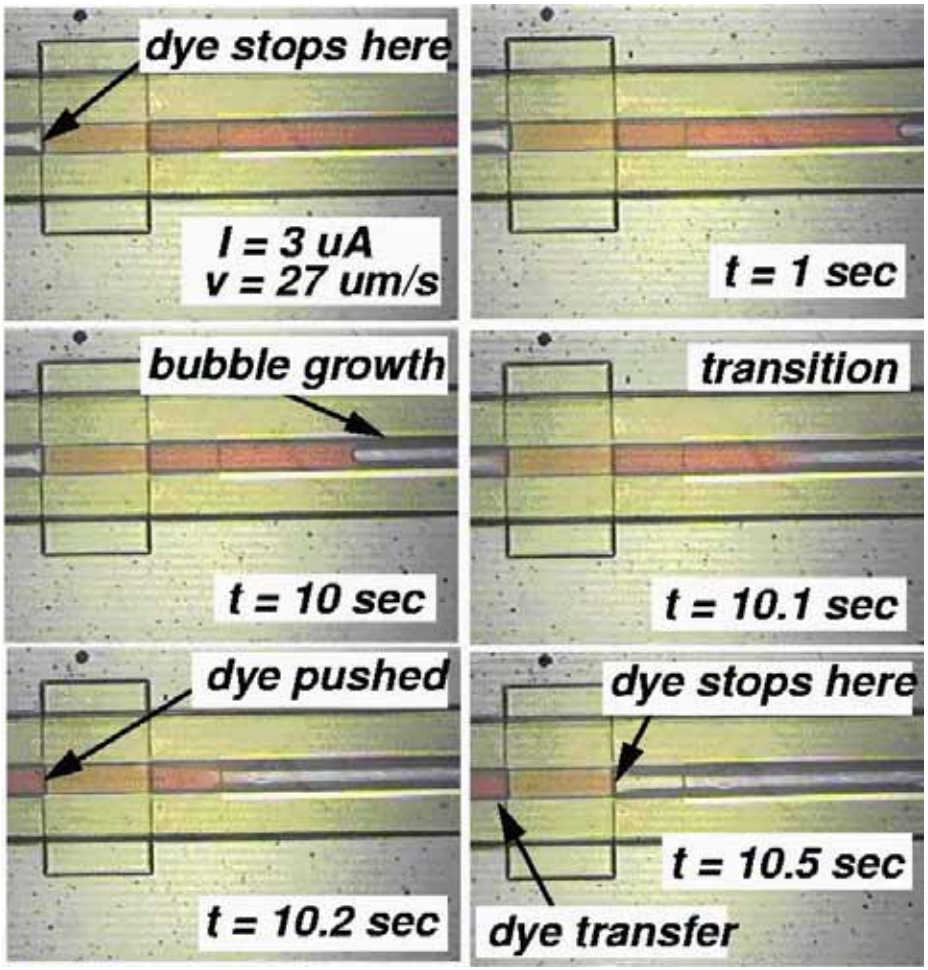


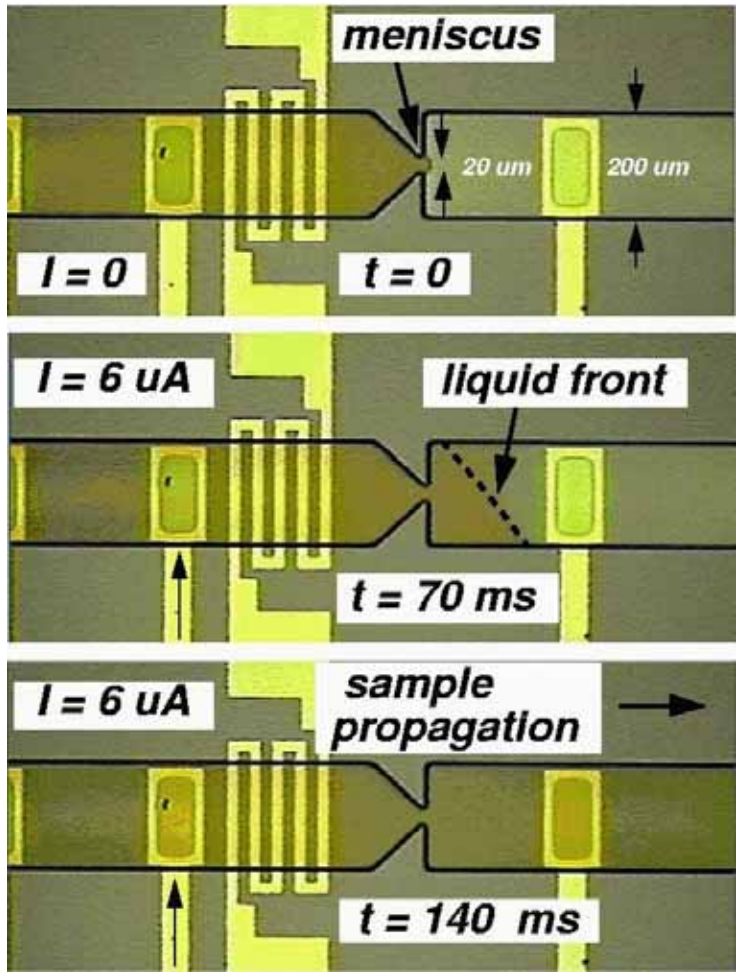



# Time Sequence Of Injector Activation

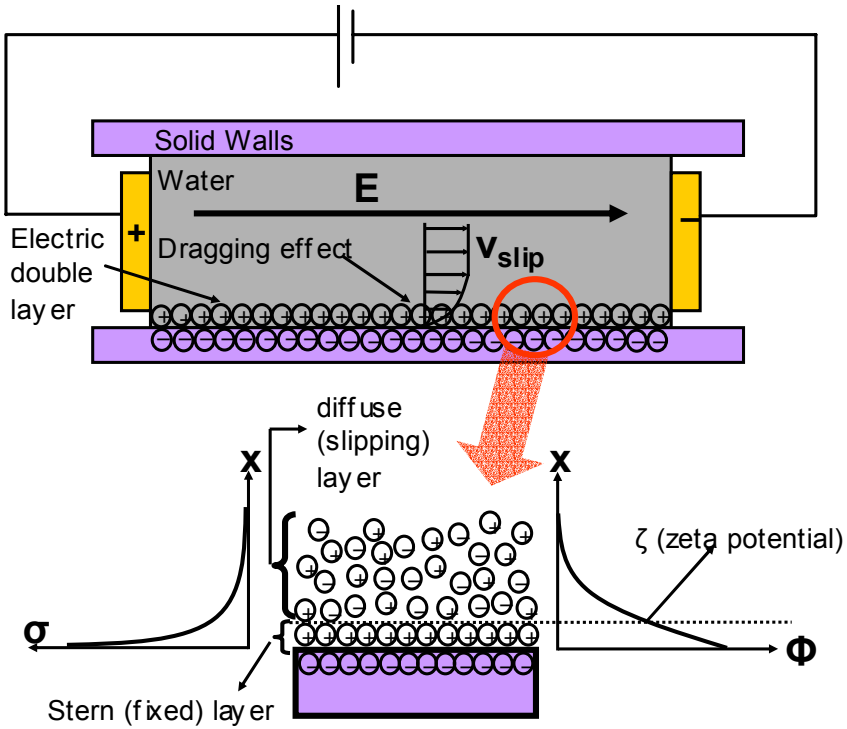



Vertical stop valve injector




Horizontal stop valve injector

# *Bidirectional Electro-Osmotic Pumping (EOP)*

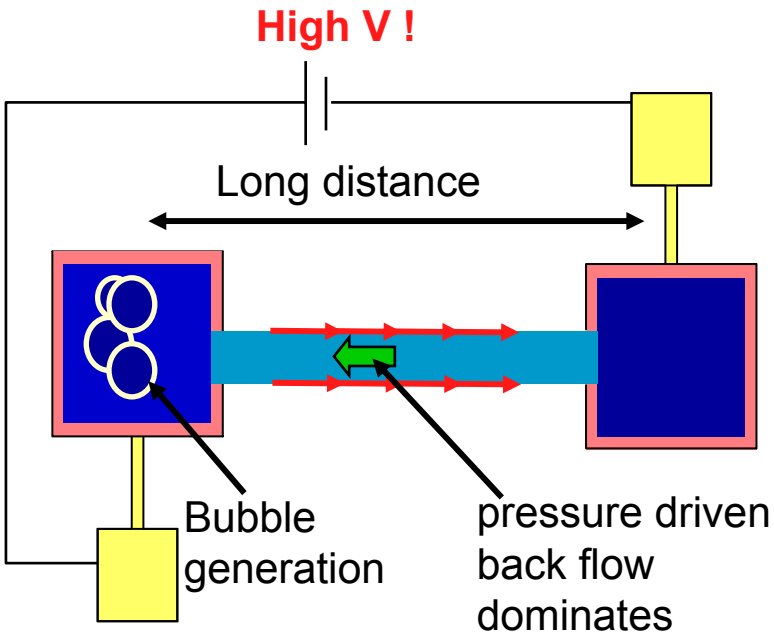



Electro-osmotic pump: Interaction between solid wall and polar liquids forms electric double layer, containing stern and diffuse layers. Diffuse layer has mobile charges where electric field moves them, dragging the bulk of the liquid with it due to viscous effects.

## Advantages of EOP

- Can pump aqueous liquids.
- Plug like movement (little dispersion while transporting)
- No moving parts
- Velocity proportional to E field
- Force proportional to surface of capillary
- Simple fabrication
- Large-scale integration compatible

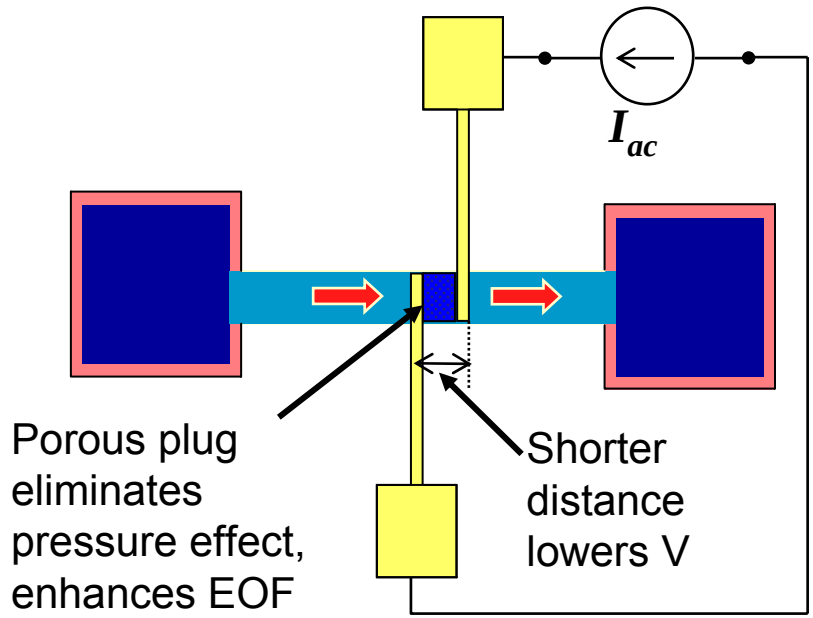
# Problems of Conventional EOP




- DC voltage drive causes electrolysis and bubbles. Thus, electrodes are put in reservoirs over long distances
- Needs high voltages if long distances (> 1000 V).
- Pressure driven back flow limits efficiency.

## *Solutions:*

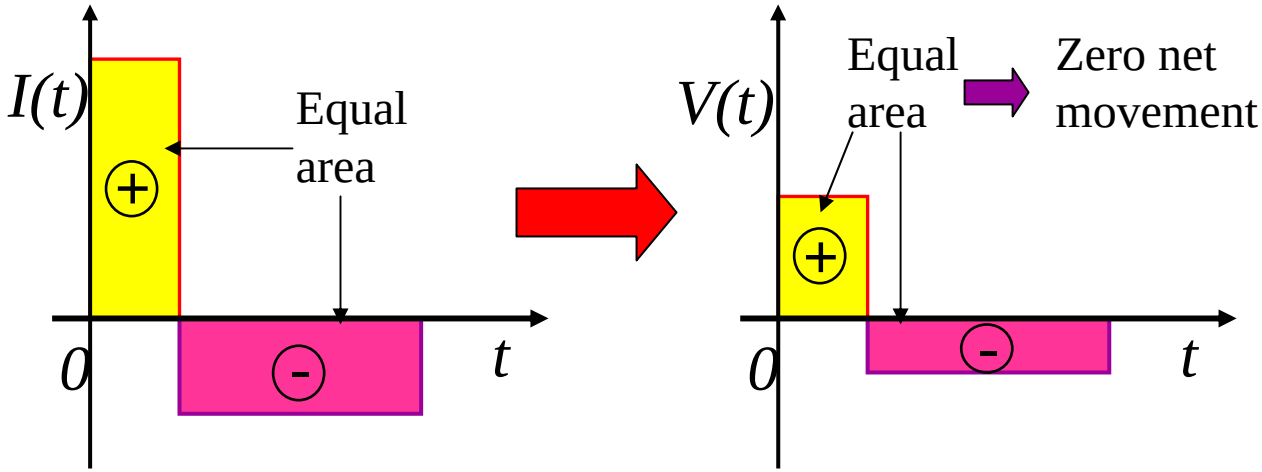
- Closely spaced electrodes
  - High field with low voltage.
- Use highly resistive porous plug
  - High surface area and high resistance to back flow
- Use AC current drive with zero net charge
  - Supress bubble generation (bf-EOF).



# AC Driven Bubble Free EOF

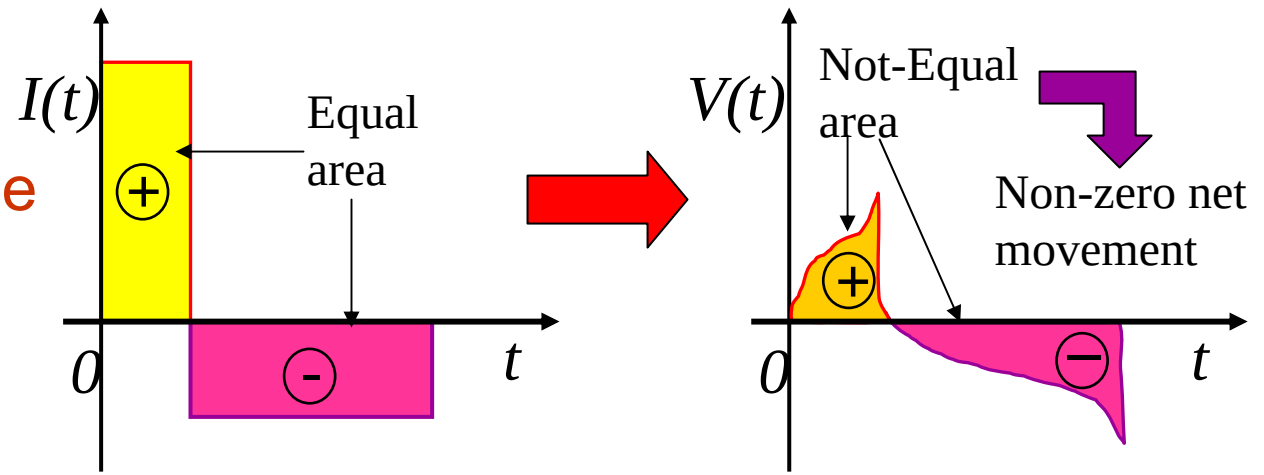
High frequency:

Electro-chemical cell produces linear voltage response.



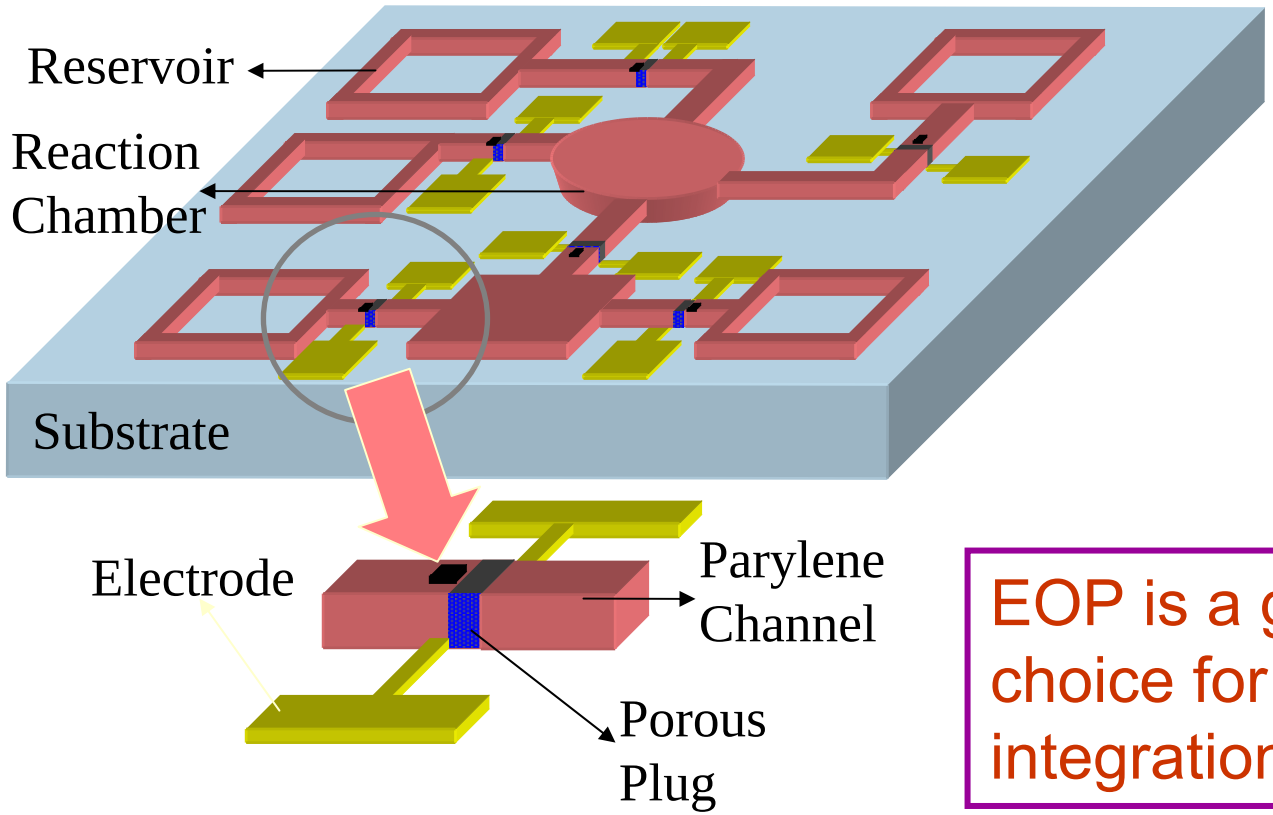

Low frequency:

Voltage response is non-linear.




P. Selvaganapathy *et al*, *J. Microelectromech. Syst.*, vol. 11, pp. 1-6, 2002

# *Bidirectional Transport Using Porous Plug Electro-osmotic Pumps*



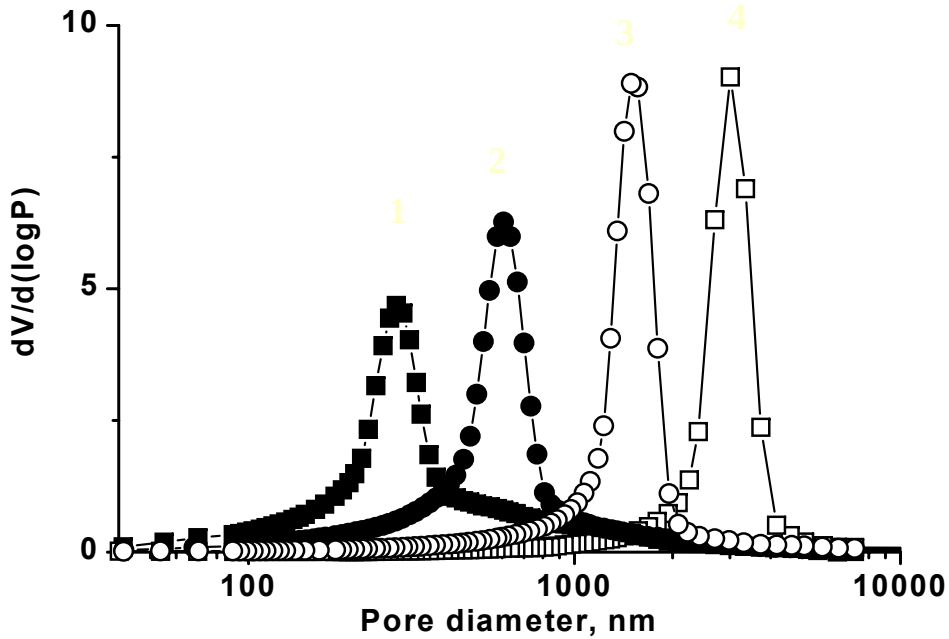

EOP is a good choice for integration

# Porous Polymer Composition

*Monolith mixture:* [1]

- ***Ethylene dimethacrylate (EDMA)***
- ***Butyl methacrylate (BMA)***
- ***2-acrylamide-2-methyl-1-propanesulfonic acid (AMPS)***
- ***Azobisisobutyronitrile (AIBN)***
- ***1-propanol***
- ***1,4-butenediol***

*Their percentages determine pore sizes precisely.*

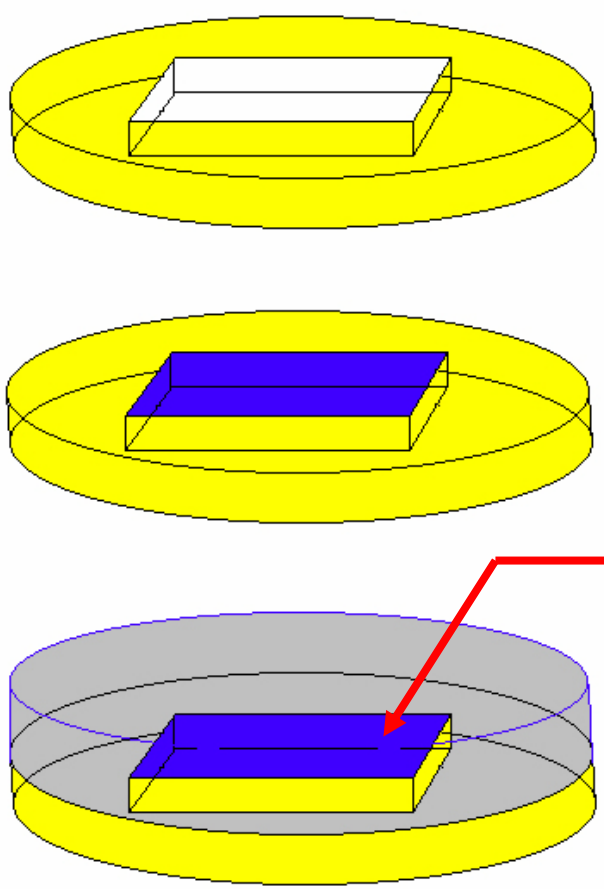



Pore size distribution profile with different 1-propanol percentages. Peak 1, 80%; 2, 78%; 3, 76%; 4,74%

[1] Reference: Cong Yu *et al*, Electrophoresis, vol. 21, pp 120-127, 2000

# Porous Polymer Micromachining

## *Forming thin film of porous polymer by casting*



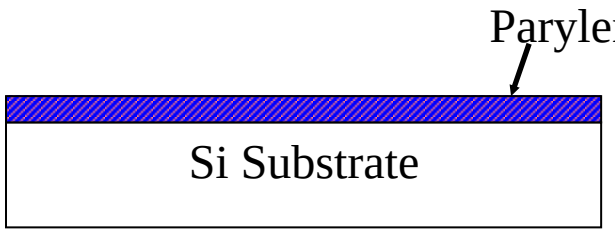
*20 µm glass well etched to hold the monomer mixture*

*Monomer solution filled into the glass well*

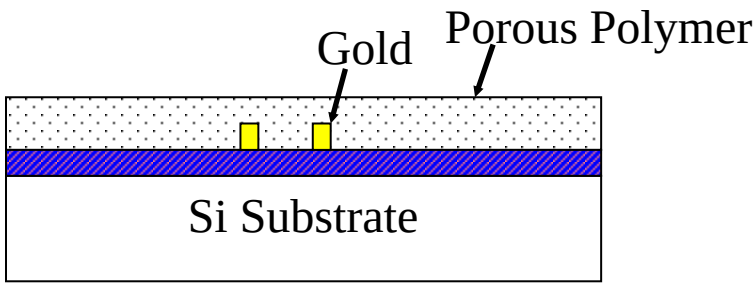
*Silicon wafer placed on top.Solution between wafers holds them together.*

*(10:10:1) DI, IPA, AZ174 (γ-metha-cryloyloxypropyl trimethoxy silane) deposited on silicon substrate in a CVD system for thin film's adhesion.*

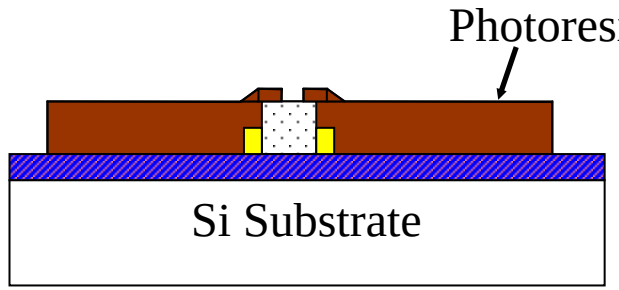
# PP-EOP Fabrication



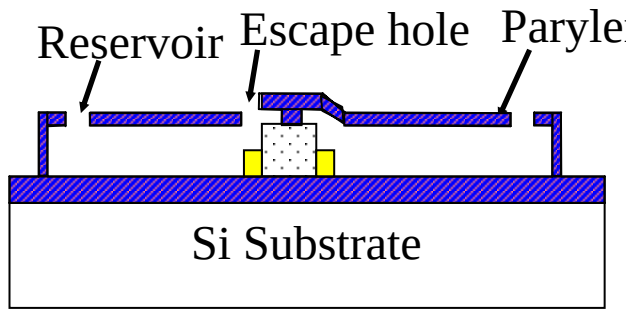

1.Deposit insulating bottom layer




2. Lithography 1- Pattern electrodes. Polymerize porous polymer.

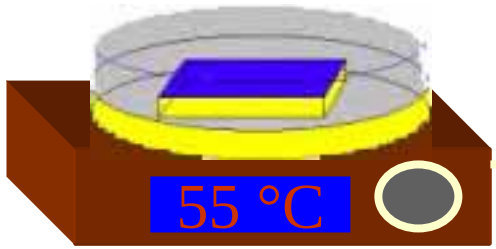



3. Lithography 2-Pattern porous plug mask. RIE etch porous polymer. Lithography 3-Pattern sacrificial photoresist.

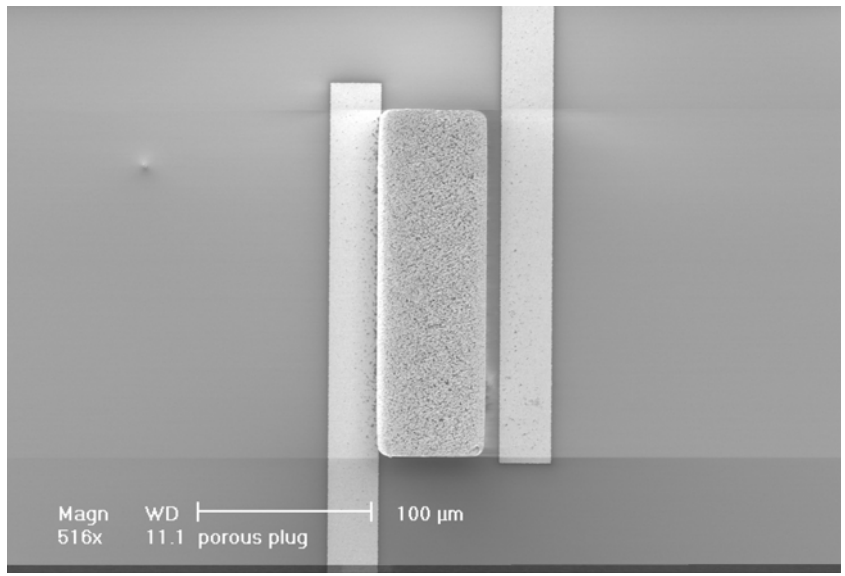



4. Lithography 4-Pattern reservoir, electrode, escape hole openings. RIE etch parylene. Acetone release sacrificial photoresist.

# Pattern Generation With RIE



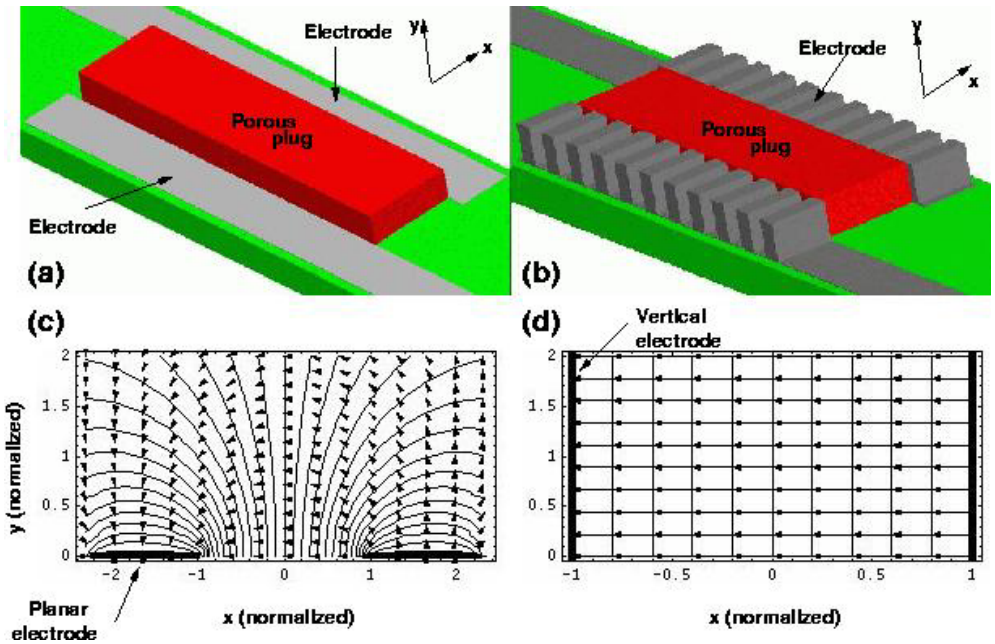

- *Polymerize over entire wafer surface overnight on a hot plate at* 55 °C.
- *Pattern a metal mask on porous polymer using lift-off process*
- *Use RIE to etch polymer on unprotected areas.*

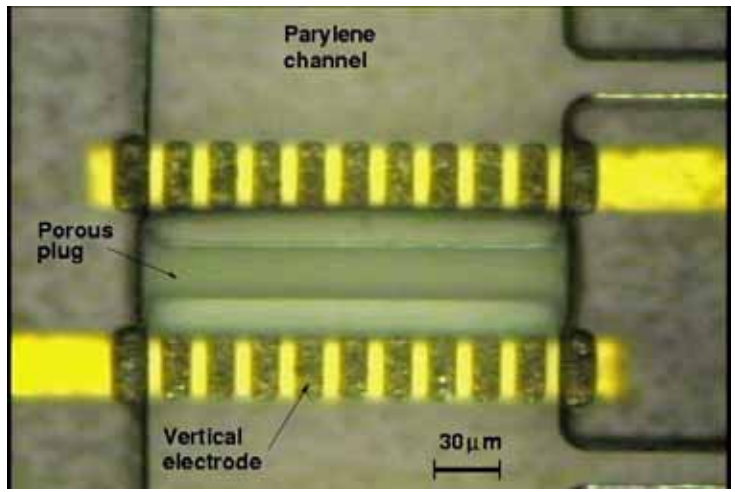



*RESULT:*

*Very high resolution. Therefore, RIE method was favored.*

# Vertical Electrodes Increase Efficiency



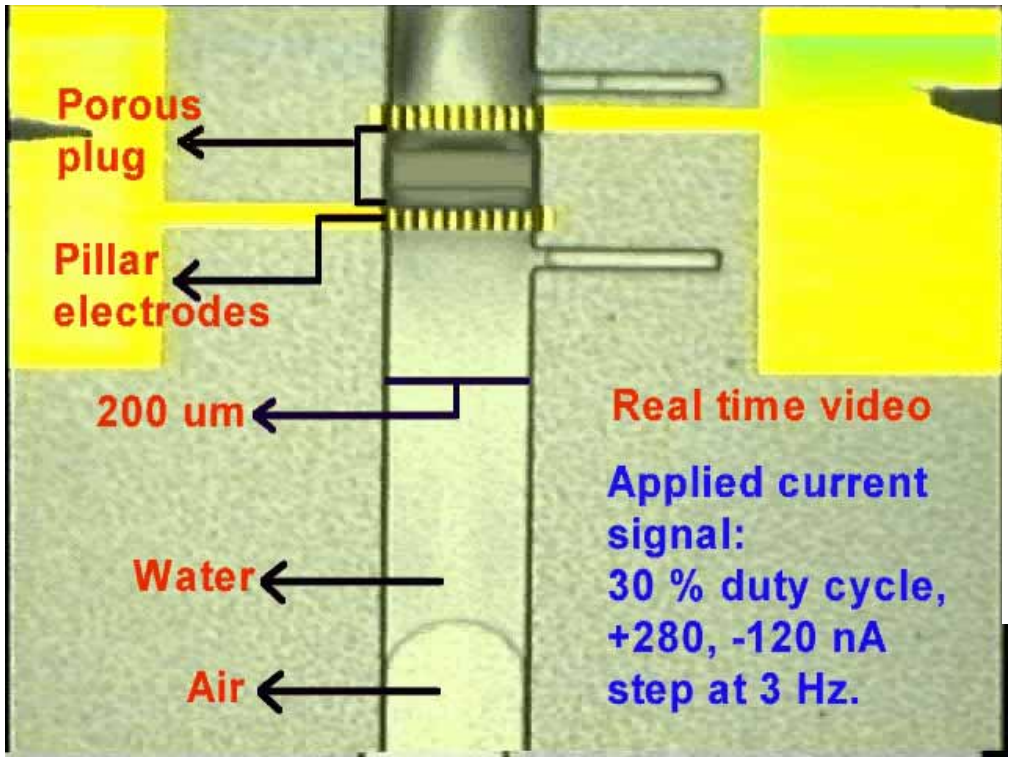

Electrode area improvement:

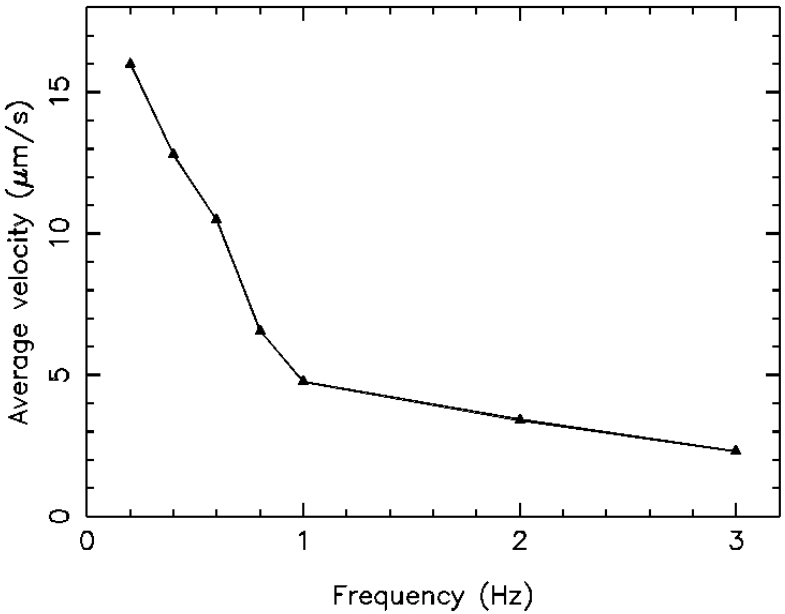
1 planar electrode area ⇒ 6000 μm²

1 vertical electrode area ⇒ 20400 μm²

Larger electrode area → Lower current density → More immunity against bubble generation

# *Velocity vs. Frequency*

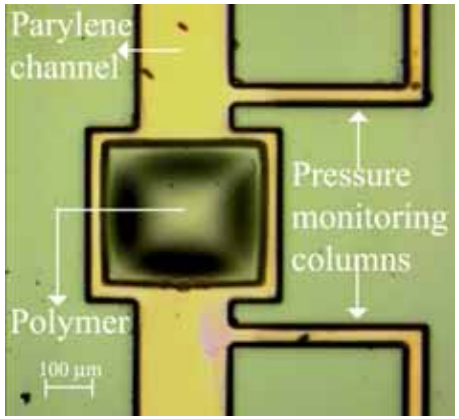


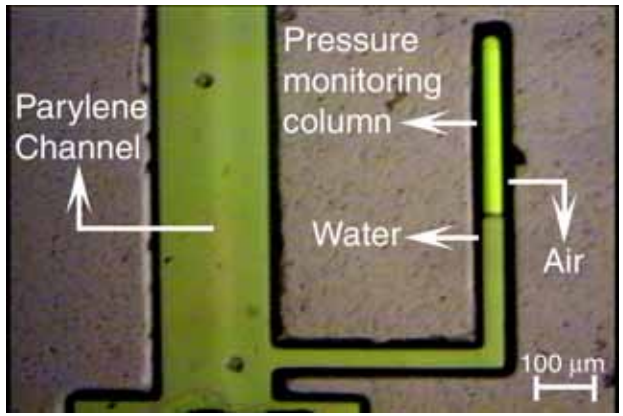



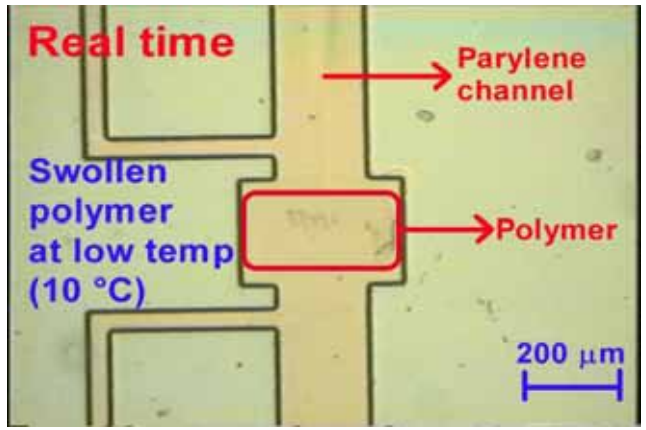

Average water-air interface velocity of the device at different frequencies.

Flow rate ≈ 3.8 nL/min → ~9 fold increase from planar electrode devices

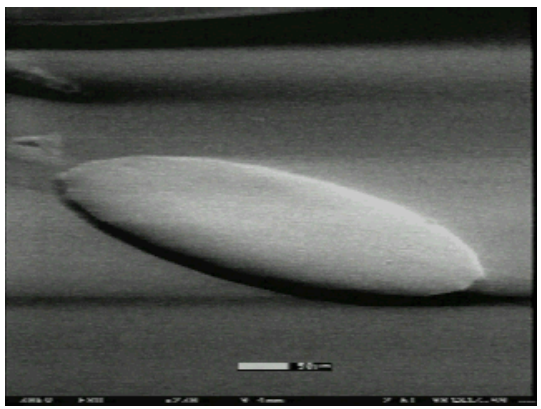
## *Photoresist Like Thermally Responsive Polymer Valve*

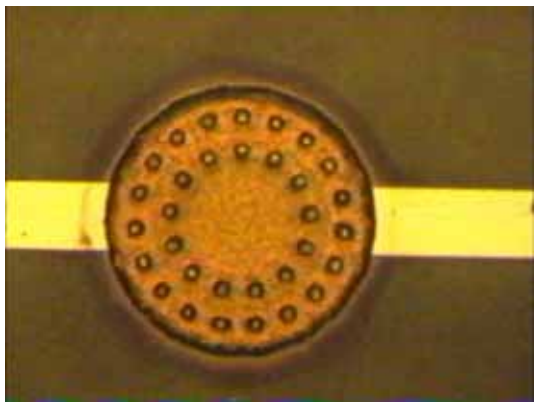


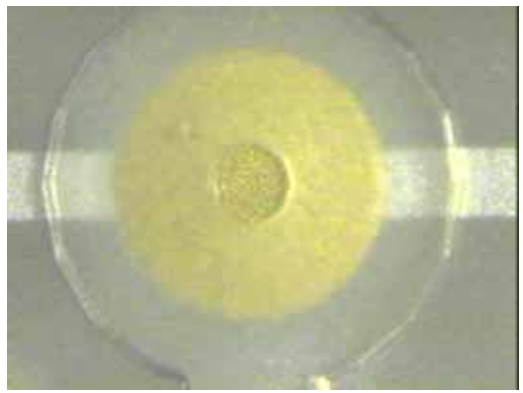




## *Waxy Polymer Actuator Valve*







# Summary

- *DNA Assays can be miniaturized on plastic microfluidic chips*
- *On-chip separations with on-chip detection demonstrated*
- *Channels, electrophoresis with detection, injectors, valves and pumps operating together on a common substrate*
- *Need library of process-compatible microfluidic components*
- *Need to think about novel simplified mechanisms to reduce system complexity*
- *Much work to be done – sample injection and lysis*
- *Handheld microfluidic DNA assays could appear in ~5-10 years.*

# Acknowledgments


- *This work is supported by DARPA BIOFLIPS, Biofluidic Chips Program, under the contract # F30602-00-1-0571 and DARPA MTO*
- *National Human Genome Research Institute (NIH-NHGRI)*